1.1. Introduction

*Crataeva nurvala*, commonly known as “Steaved Tree” in English and “Varuna” in Sanskrit, is a moderate sized deciduous tree. Bark is grey, smooth, horizontally wrinkled and leaves are trifoliate. Flowers are white or creamy, in many flowered terminal corymbs. Fruits are multiple seeded and ovoid berries. Seeds are embedded in yellow fleshy pulp. Whole of the plant is used for medicinal purpose.1-3

Mature bark is generally used for extraction which is 6-15 cm long, 3-10 cm wide with thickness varying from 5-12 mm. Outer surface grayish - brown colored & rough due to presence of numerous small & rounded lenticels & transverse wrinkles. Inner surface smooth & whitish brown to buff colored. The water extract also contains tertiary and quarternery bases including choline. The leaves yield flavonoids including rutin, quereetin and isoquer­­cetin. The water extract of varuna bark reportedly, displays nicotinic actions on guinea pig ileum and dog tracheal muscle *in vitro*. The alcohol / ether extract of the bark has anti-inflammatory activity comparable to that of corticosteroids. Acute inflammations induced by histamine and carrageenin, and delayed (chronic) inflammations (in formaldehyde induced arthritis) are both inhibited. The water extract of varuna leaves displays antibacterial activity against *Shigella* and *Salmonella* *in vitro*. Use of varuna decoction on bladder emptying, chronic urinary infection and urinary electrolyte excretion is well known. Varuna decoction reduces the urinary excretion of sodium and magnesium, shifting values from the lithogenic to the non-lithogenic zones. It was also found to increase the 'spontaneous' passage of renal and bladder calculi. In patients with prostatic hypertrophy, varuna decoction increases the force of detrussor contraction, reducing the volume of residual urine. This action, through which stagnation of urine is reduced, combined with its anti-inflammatory property, may explain the antiseptic action of varuna on the urinary tract. In ayurvedic practice, varuna is used as a litholytic agent, in treating 'Kapha' and 'Vata' varieties of ashmari (calculi). It also clears crystalluria. It is the drug of choice in all kapha disorders of the urinary tract, and in renal and bladder calculi. It is also used as a cholagogue, anti-helmintic and antiameobic in both intestinal and hepatic infestation.3 Its action as an anti-inflammatory agent is utilized in small joint diseases and in osteomyelitis. It is also used as an antipyretic. The vesicant effect of topical application of freshly ground varuna leaves has been mentioned in ayurvedic literature.
Lupeol and Varunol (Triterpenoids) have been isolated from root and stem bark of Crataeva nurvala. Major component of stem-bark is Lupeol. Other than Lupeol, lupenone, and β sitosterol etc are the other minor components present in stem bark of this plant. Lupeol, Lupeol acetate, α-spinasterol acetate, α-taraxsterol, 3-epiLupeol, and β-sitosterole are the major constituents and β-sitosterone acetate are the minor constituents isolated from root bark of *Crataeva nurvala*. Lupeol shows inhibitory effect on various inducible enzymes, such as nitric oxide synthase (NOS) & cyclo-oxygenase-2 (COX-2) enzymes, Protein kinase (PKA) & Serine Proteases etc. It shows a large number of highly interesting biological actions which are discussed under the following heading:-

- **Anticancer** – Lupeol (from mango pulp extract) was found to be effective in combating oxidative stress induced cellular injury of mouse liver by modulating cell-growth regulators. Lupeol hence could be a potential against pancreatic cancer. Lupeol was found to exhibit antitumor-promoting effects in CD-1 mouse skin tumorigenesis model. The animals pre-treated with Lupeol showed significantly reduced tumor incidence, lower tumor body burden and a significant delay in the latency period for tumor appearance, against 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced skin tumor promotion. It exhibited in vitro cytotoxicity against human hepatocellular carcinoma (Hep-G2) and human epidermoid carcinoma (A-431), while did not affect the growth of tumor cell lines such as human melanoma (MEL-2), human lung carcinoma (A-549), and murine melanoma (B16-F10). Moreover, Lupeol was found to exhibit a significant antiangiogenic activity on in vitro tube formation of human umbilical vein endothelial cells (HUVEC). In addition, induction of apoptosis by Lupeol was observed in human leukemia HL-60 cells.

- **Antimalarial** – Long chain Lupeol fatty ester showed showed inhibitory activity against drug resistant strains of *P. falciparum* at a dose of 1.02-18.53 μg/mL. Lupeol exhibits inhibitory activity on *P. falciparum* growth in vitro but lacks in vivo activity in mice infected with *P. berghei*. Lupeol causes membrane shape changes of erythrocytes toward stomatocytic forms observable at concentrations below its IC₅₀ (11.8 μg/mL or 27.7 μM). Another plant *Vernonia brasiliana* extract, having antiplasmodium activity in vitro in which Lupeol was identified as a compound responsible for the activity. The structure of lupeol is reminiscent of that of cholesterol, and the compound is expected to be able to enter the cellular membranes. Due to the presence of a single hydroxy group and a large, apolar skeleton, and lupeol acts as an amphiphile. According to the bilayer
hypothesis Stomatocytes are generally formed when a lipophilic compound is incorporated into and expands the inner layer of the lipid membrane.\textsuperscript{15,16} Screening of the 96-member library for in-vitro antimalarial activity against \textit{P. falciparum} in vitro led to the identification of compounds with seven to nine fold increase in the biological activity in comparison to Lupeol.\textsuperscript{17}

- **Antimicrobial** – Lupeol has also been used for fungal infection in human. It may be administered intravenously or intraperitoneally by infusion or injection.\textsuperscript{18,19} Lupeol derivatives like Lupenone, Lupeol acetate have also been found to show antifungal activity. Lupenone showed antifungal activities against \textit{Saccharomyces cerevisiae} and \textit{Microsporum gypseum}\textsuperscript{20} and \textit{Phytophthora cactorum}. Lupeol acetate isolated from \textit{Mikania monagasensis} was found effective against \textit{Staphylococcus aureus} and \textit{Candida albicans}.\textsuperscript{21} Lupenone were evaluated for their antiviral activities against Herpes Simplex Virus and African Swine Fever Virus. Lupenone exhibited strong viral plaque inhibitory effect against HSV-1 and HSV-2.\textsuperscript{22,23}

- **Anti-inflammatory** – Lupeol (50mg/kg) and Lupeol Acetate (50 mg/kg) were investigated for there anti-inflammatory activity\textsuperscript{24-26} in comparison with the commonly used non-steroidal anti-inflammatory drug, indomethacin (3 mg/kg) in rats. The presence of anti-inflammatory activity in Lupeol & Lupeol acetate seems interesting, since they possess hydro-aromatic ring systems more or less similar to that of steroid and devoid of side-effects. Lupeol-3-palmitate and Lupeol-3-linoleate, two synthetic long chain fatty acid ester analogues of Lupeol were studied \textit{in vitro} as potential inhibitors of serine protease activity.\textsuperscript{27,28} With respect to the natural protein substrate bovine serum albumin, Lupeol palmitate and Lupeol linoleate inhibited trypsin activity in a manner consistent with mixed inhibition (K\textsubscript{IC} values of 103 and 52 $\mu$M respectively; K\textsubscript{IU} values of 30 and 14 $\mu$M respectively). Effect of Lupeol and its ester have been reported to be due to its effect on keratynocyte proliferation especially by its hemisuccinylation.\textsuperscript{29} The Lupeol in the doses of 200, 400 and 800 mg/kg produced a dose dependent inhibition \textit{i.e.} 24%, 40% and 72% where as 19 $\alpha$-H- Lupeol showed 21%, 47% and 62% inhibition after 24 h in acute model of inflammation.\textsuperscript{30}

- **Antiarthritic** – Lupeol linoleate and indomethacin showed a reduction in paw swelling by 39, 58 and 35%, respectively, in adjuvant arthritis.\textsuperscript{30,35} In arthritis model, Lupeol exhibited 29% and 19 $\alpha$-H- Lupeol 33% inhibition after 21 days respectively.\textsuperscript{28} Lupeol and its ester are reported to reduce the alteration induced in arthritis animals like
significant increase in the level of lipid peroxide, superoxide, dismutase glutathione peroxidase and catalase.\textsuperscript{36} It has been found to suppress various immune factors such as the phagocytic (cell killing) activity of macrophages, T-lymphocyte activity that included CD4+ T cell mediated cytokine generation. Oral administration of Lupeol at doses of 12.5 – 200 mg/kg p.o. inhibited CD4+ T and CD8+ T cell counts and cytokines IL-2, IFN-\(\gamma\)- (Th1) and IL-4 (Th2).\textsuperscript{37} Lupeol and its ester have also shown remarkable activity in the chronic cotton pellet granuloma and Freund’s adjuvant arthritis model.

- **Hepatoprotective** – Lupeol & its ester derivative Lupeol linolate were investigated for their possible hepatoprotective effect against cadmium induced toxicity in rats. Lupeol linolate showed a better effect on the antioxidant status of the liver.\textsuperscript{38} Chemopreventive properties of Lupeol were evaluated against 7, 12-dimethylbenz (a)anthracene induced alteration in liver of Swiss albino mice. Lupeol (25 mg/kg body weight) were daily given once for 1 week after a single dose of 7,12-dimethylbenz(a)anthracene 60 mg/kg). Lupeol supplementation effectively influenced the 7,12-dimethylbenz(a)anthracene induced oxidative stress, characterized by restored antioxidant enzyme activities and decrease in lipid peroxidation. A reduction of apoptotic cell population in the hypodiploid region was observed in Lupeol supplemented animals. Thus, Lupeol was found to be effective in combating oxidative stress induced cellular injury of mouse liver by modulating cell-growth regulators. It was observed that pretreatment with Lupeol (100 mg/kg body mass, orally) and silymarin (100 mg/kg body mass, orally) for 7 days reverted the condition to near normalcy in aflatoxin B-1 administered rats.\textsuperscript{39}

- **Antioxidant** – Lupeol showed the nephroprotective action in rat kidney after chronic cadmium exposure. Cadmium at a dose of 1 mg kg\textsuperscript{-1} body weight was administered subcutaneously for 15 days to rats. This led to an increase in the level of lipid peroxides and a decrease in the level of antioxidants in the kidney. The efficacy of Lupeol, when supplemented at a dosage of 40 mg kg\textsuperscript{-1} body weight concurrent with cadmium administration, showed an improvement in the antioxidant status.\textsuperscript{40} Lupeol is also reported as a strong antioxidant protection against benzoylperoxide induced toxicity in Swiss albino mouse skin.\textsuperscript{41} Lupeol administration induced a remarkable decrease in kidney oxalate level and also was effective in counteracting the free radical toxicity by bringing about a significant decrease in peroxidative levels and increase in antioxidant status, thereby exerting cytoprotective action against free radical damage during stone formation.\textsuperscript{42}
• **Hyper Cholesterolemia** – Lupeol and Lupeol linoleate decreased the lipid peroxidation levels and increased enzymatic and nonenzymatic antioxidants also ameliorating the lipidemic–oxidative abnormalities in the early stage of hypercholesterolemic atherosclerosis. Lupeol and its derivative showed significant increase (\( p < 0.05 \)) in lipid peroxidation was paralleled by significantly diminished (\( p < 0.05 \)) activities of antioxidant enzymes (SOD, CAT and GPx) and decreased (\( p < 0.05 \)) concentration of antioxidant molecules (GSH, Vit C and Vit E) in cardiac tissue and hemolysate of high cholesterol diet fed male albino rats.43

• **Urinary disorder** – Crude extract of *Crataeva nurvala* (stem bark) was found to exhibit antiurolithiatic activity, in which Lupeol was found as an active constituent. Antiurolithiatic activity of Lupeol44 was assessed in rats by observing the weight of the stone, biochemical analysis of serum and urine, and histopathology of bladder and kidney. It has the ability to decrease the levels of peroxidative damage in calcium-oxalate-induced urolithiasis in rats. Administration of Lupeol to hyperoxaluric rats (25 mg/kg body weight/day) reduced significantly the renal excretion of oxalate. It also reduced the extent of renal tubular damage as evidenced from the decreased levels of the various enzymes in urine.

• **Antiulcer** – Esterified Lupeol (Lupeol acetate) was studied for its antiulcer activity. Lupeol acetate decreased the incidence of gastric ulceration induced by pyloric ligation45 2-substituted derivative of lupenone also showed antiulcer activity.

• **Antifertility** – The treatment with Lupeol acetate at the dose level of 10 mg/rat/day cause significant induction of antifertility in male albino rats. Biochemical parameters of tissues i.e. protein, sialic acid, glycogen and cholesterol content of testes and seminal vesicular fructose has also shown significant reduction.46

• **Other activity** – Various other activities have been reported from the Lupeol such as Lupeol along with betulin shows cough suppressant and expectorant effects47 Lupenone showed antibacterial activity against *E.coli* and *Staphylococcus aureus*. Lupeol hemisuccinate, a ester derivative is useful for cosmeceutical applications. Lupeol and its esters with different organic acids have been employed as the constituents of a skin aging-preventing cosmetic lotion, which promotes keratinocyte proliferation and give moisture smoothness and lustre to skin.48,49
1.2. Present status and need of new antimalarials

Malaria is an infectious disease of enormous important, affecting over 100 countries of the tropical and subtropical regions of the world. *Plasmodium falciparum*, the causative agent of the most malignant forms of malaria, is a parasite lethal for the infected patients. Despite of tremendous efforts an effective vaccine has not been developed yet. Emergence and spread of the rapid resistance toward current drugs including chloroquine encourages the study for new active molecules. Hence there is an urgent need to have a new antimalarial agent with different structural prototype.

Traditional remedies have always been a source of important antimalarial drugs\(^{50,51}\) and continue to provide novel and effective treatments. Among the diseases traditionally treated with medicinal plants, malaria is one that deserves special attention first of all, because it is one of the most important tropical diseases, killing more people than any other communicable disease except tuberculosis and AIDS (WHO, 2001). Around 300-500 million clinical cases of malaria are reported every year, recent survey estimated to cause more than 2 million deaths annually\(^{52-55}\) among which half are children under five years old.\(^{56}\) Every 40 seconds a child dies of malaria, resulting in a daily loss of more than 2000 young lives worldwide. Although it is in principle preventable and curable, the majority of the population at risk cannot afford to pay for modern drugs so that there is an urgent need for affordable treatments.

The aims of using drug in relation to malarial infection are –
1. To prevent and treat clinical attack of malaria.
2. To completely eradicate the parasite from the patients body.
3. To reduce the human reservoir of infection:- cut down the transmission to mosquito.

These are achieved by attacking the parasite at various stages of life cycle in human host.

1.2.1. Life cycle of malaria parasite

Malaria is caused by protozoan parasites, namely *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, and is transmitted to humans by female mosquitoes of the genus *Anopheles*. In India five species of *Anopheles* are known to be transmitters of the parasites: (1) *A. culicifacies*, (2) *A. stephensi*, (3) *A. philippinensis*, (4) *A. sundaicus*, and (5) *A. fluviatilis*. Endemic maps indicate that *P. falciparum* and *P. vivax* account for 95% of malaria infections.\(^{57}\) The human malaria parasite has a complex life cycle. Being digenetic, it is completed in two hosts. The asexual cycle is passed in humans by a process termed
schizogony. The sexual cycle is completed in the final host or vector, the female *Anopheles* mosquito, involving gametogony and sporogony. The infection starts when the infected female *Anopheles* mosquito injects sporozoites into the subcutaneous tissue (1) and less frequently directly into the blood stream; from there sporozoites travel to the liver. This is followed by a period of incubation during which the sporozoites invade the hepatocytes where they grow and multiply (2). Towards the end of the incubation period, tissue schizonts rupture to release merozoites (3), which enter into the blood stream and infect the erythrocytes (4). Within the erythrocyte parasite grows from "ring" to mature trophozoite, then to schizont, and finally to merozoite. These merozoites are released into the blood stream by the rupture of erythrocytes (5). This is accompanied by the manifestation of the clinical symptoms of the disease such as fever, shivering and anaemia. Merozoites reinfect the healthy erythrocytes. Some of the merozoites are changed into gametocytes (6) that are ingested by female *Anopheles* when it bites an infected person. In female mosquito these gametocytes undergo sexual reproduction and produce sporozoites. These sporozoites migrate in the salivary glands; from there they are injected into the blood stream of a healthy person. In this way the infective cycle of the malaria parasite continues.

*Figure 1: Life Cycle of Malaria Parasite*
1.2.2. Chemical classes of antimalarials and their present status-

Malaria was among the first diseases to be treated with a pure chemical compound. Antimalarial drugs can be classified by the chemical group to which they belong which in turn determines the stage of parasite life cycle they effect. Principal drugs and different classes of compounds are briefly discussed below:

- **Quinolines**

  **4-Aminoquinolines** Chloroquine \( \text{I} \) (CQ) synthesized in 1934, became the drug of choice in several programs aimed at the global eradication of malaria. Chloroquine \( \text{I} \) rapidly acts on blood schizontocides and also has some gametocidal activity.\(^58^{,}59^{,}60\) It remains the best drug for a long time because of excellent clinical efficacy, few side effects and cost effective synthesis. It acts by inhibiting heme polymerization. Unfortunately most strains of *P. falciparum* malaria are now resistant to this drug.\(^61\)

  **4-Methanolquinolines** Mefloquine \( \text{II} \) are aryl amino alcohols, which rapidly acts on blood schizontocides against all the species of malaria. It destroys the trophozoites present in the erythrocytes but has no effect on the *exo-* erythrocytic stages that develop in the liver.\(^62\) Mefloquine appears to act by inhibiting heme polymerase. It is selectively active against the intra erythrocytic mature forms (trophozoites and schizonts) of malaria and has no activity against mature gametocytes.\(^58\) Both *in vitro* and *in vivo* resistance has been reported against mefloquine in malaria endemic regions.\(^59\)

  **8-Aminoquinolines** Primaquine \( \text{III} \) a tissue schizontocides is used in the radical curative treatment and given orally to eradicate the liver hypnozoites of *P. vivax* and *P. ovale*.\(^58^{,}59\) The use of this vital 8-amino quinoline is imposing great limitations because of inherent side effects like intravascular haemolysis in glucose-6-phosphate dehydrogenase deficient cases, anaemia and methaemoglobin toxicity.\(^63^{,}64\)

Besides these resistance of *P. vivax* to primaquine \( \text{III} \) has also been reported in several parts of the world.
• **Antifolates**

**Diaminopyrimidines** includes compounds like 2,4-diaminopyrimidine derivative pyrimethamine IV and trimethoprim V. They inhibit the activity of dihydrofolate reductase & are active against asexual blood stages of all types of malaria and also active against primary exoerythrocytic stages.\(^{59,62,65}\) Both of them are always given in combination with either a sulphonamide or sulfone.

**Biguanides** includes proguanil VI an active cyclic triazine metabolite is the best compound of this series. The antimalarial activity of proguanil was ascribed to cycloguanil VII, shown to be selective inhibitor of the bifunctional plasmodial dihydrofolate reductase – thymidylate synthetase thereby inhibiting DNA synthesis and depleting folate cofactors.\(^{66,67}\) Proguanil is effective against the primary exoerythrocytic forms of *P. falciparum* and asexual blood forms of all species of human malaria parasite.\(^{62,68}\)

• **Sulphonamides and Sulfones**

These include sulphadiazine VIII, sulphadoxine IX, sulphalene X, dapsone XI and acedapsone XII. Sulphonamides and sulfones are slow acting blood schizontocide that are more active against *P. falciparum* than *P. vivax*. These compounds are used in combination with dihydrofolate reductase inhibitors to enhance their antiplasmodial action.\(^{69}\)

The antifolate combination of pyrimethamine v and sulphadoxine has been used extensively for prophylaxis and suppression of human malaria especially those with chloroquine *P. falciparum* strains. Recent reports have shown that *P. falciparum* has developed resistance against antifolates and sulphonamides have found to show serious toxicity in some individuals.\(^{69,70}\)
Antibiotics-

Doxycycline XIII and azithromycin XIV are semisynthetic azalide antibiotics and important compounds of this class. Doxycycline is a tetracycline, which inhibits parasite mitochondrial protein synthesis. It is an effective agent when used prophylactically against multidrug resistant *P. falciparum.*

Natural compounds with antimalarial activity

Malaria is one of the diseases for which even today not many suitable drugs are available. Actually, the two most effective drugs for malaria, quinine and artemisinin, originate from plants; it is probable that other plants contain as yet undiscovered antimalarial substances. Recently several molecules isolated from natural sources have shown antimalarial activity.

Alkaloids-

**Quinoline alkaloids**

Quinine XV was isolated in 1820 as the active ingredient, from the bark of American Chinchona tree and used for the treatment of severe and complicated malaria in most parts of the world. It is a blood schizontocide, which destroys the blood trophozoites present in the erythrocytes but has no effect on the exo-erythrocytes stages developed in the liver. Mild side-effects are common in quinine XV, notably cinchonism (tinnitus, hearing loss, dizziness, nausea, uneasiness, blurring of vision).

**Indolquinoline alkaloids**

Cryptolepine XVI is an indolquinoline alkaloid isolated from the roots and leaves of *Cryptolepis sanguinolenta.* It showed in vitro activity with IC$_{50}$ 0.134µM, against a multidrug resistant strain of *P. falciparum* (K1) and in vivo activity against rodent malaria when given orally, but not subcutaneously.

**Quinazolone alkaloids**

Febrifugine XVII was isolated from Chinese medicinal plant *Dichroa febrifuga.* The compound has been found active when given orally at 1.25 to 5.0 mg/kg/day in
murine malaria models but fatally toxic in the dosage over 10 mg/kg/day.\textsuperscript{74}

**Tetrahydroisoquinoline alkaloid** Hervelines A-D (XVIII-XXI), were isolated from *Hernandia voyronii*. Compounds XX-XXI showed moderate in vitro antimalarial activity (IC\textsubscript{50} = 1.68 to 3.38 \(\mu\)g/mL) against the CQ-resistant *P. falciparum* strain.\textsuperscript{75}

\begin{align*}
\text{XVIII} & \; R_1 = \text{H}, R_2 = \text{CH}_3 \\
\text{XIX} & \; R_1 = \text{CH}_2, R_2 = \text{H} \\
\text{XX} & \; R_1 = \text{CH}_3, R_2 = \text{CH}_3 \\
\text{XXI} & \; R_1 = \text{H}, R_2 = \text{H}
\end{align*}

**β-carbol ine alkaloids** Manzamine A XXII present in several marine sponge species, inhibits the growth of *P. berghei* in mice. More than 90% of the asexual erythrocytic stages of *P. berghei* were inhibited after a single i.p. injection of XXII (50 \(\mu\)g/mL) into infected mice. A remarkable aspect of XXII treatment is its ability to prolong the survival of highly parasitemic mice, with 40% recovery 60 days after a single injection.\textsuperscript{76}

- **Amino steroids**

Sarachine XXIII an amino steroid isolated from the leaves of the shrub *Saraca punctata* (Solanaceae). The compound showed a strong in vitro antiplasmodial activity with an IC\textsubscript{50} of 25 nM.\textsuperscript{77} Compound XXIII was also active *in vivo* against *P. vinckei*, with an 83% inhibition of parasitaemia at 100 mg/kg per 2 days.

- **Chalcones**

Licochalcone A XXIV is isolated from the roots of Chinese liquorice.\textsuperscript{78} This compound showed significant antimalarial activity in vitro against 3D7 and Dd2 strains of *P. falciparum* and in vivo against *P. yoelii* when administered intraperitoneally.\textsuperscript{79} Chalcones act by inhibiting cysteine protease, which degrades hemoglobin to the amino acids needed for growth by the parasite.\textsuperscript{80}
• Xanthones
Ruficallol XXV, a hydroxylated anthraquinone, showed in vitro activity with an IC$_{50}$ of 35 nM initially and latter exhibited an IC$_{50}$ of 226 nM both against a CQ-sensitive strain (D6) of *P. falciparum*. Exiphone XXVI, showed in vitro activity with an IC$_{50}$ of 4.1 µM against CQ-sensitive strain (D6) of *P. falciparum*. However, combination studies have shown that XXV potentiated the antimalarial activity of XXVI. The antimalarial activity of xanthones has been linked to their ability to inhibit heme polymerization.$^{78}$

Naphthoquinones Diospyrin XXVII, isolated from *Diospyros montana* and its semisynthetic derivatives have been investigated for their antiprotozoal effects. Diospyrin tetrahydroxy derivatives XXVIII were 100-fold more active than diospyrin against the multidrug resistant *P. falciparum* K1. These compounds were as active as chloroquine with an IC$_{50}$ of 0.215 µM.$^{83}$

Anthraquinones The prenylated pre anthraquinone vismione H XXIX, isolated from *Vismia guinensis* showed antimalarial activity with an IC$_{50}$ of 0.088 µg/mL against CQ sensitive strain NF-54 of *P. falciparum*, at a level comparable to quinine.$^{84}$

• Terpenes
Endoperoxide sesquiterpenes lactone artemisinin XXX (IC$_{50}$ 0.14µM) and its derivatives artemether XXXI, artether XXXII, artesunate XXXIII etc are strong blood schizontocidal against *P. falciparum*, and are the best antimalarial drugs of
Artemisinin derivatives do not induce hypoglycemia in pregnancy, although there is still very little information on their use in pregnancy.

The efforts to eradicate malaria have failed due to emergence of parasites resistant to conventional drugs, imposing a lot of pressure on public health systems to introduce new treatments. Using the effective drug against the particular parasite strain in the affected area can solve this problem. In some areas combination therapy may be helpful where resistance against a single drug has been reported. Artemisinin and its derivatives are effective antimalarials against which no resistance is reported in clinical cases. There will always be a need for new antimalarial agents, particularly those that may have a novel mode of action. For this, a large number of plant and marine samples are required to be screened, which can provide several new leads.

1.3. Origin of Lupeol in plants

Lupane series of triterpene is a widespread group parallel to several other types of triterpenes in nature. Thus it will be relevant to discuss the formation of Lupeol in plants. Terpenoid is referred to as isoprenoid compounds. Major classes of isoprenoids are as follows: - hemiterpene - C5, monoterpenes - C10, sesquiterpenes - C15, diterpenes - C20, sesterterpenes - C25, triterpenes - C30, tetraterpenes - C40, polyterpenes - (C₅)n, etc having 1, 2, 3, 4, 6, 8 and n isoprene units respectively. These classes are further subdivided into groups based on various structural features eg. monoterpenoids can be subdivided into acyclic, monocyclic and bicyclic compounds. Triterpene itself is also a large and structurally diverse group of terpenoids, derived from squalene or related acyclic 30-carbon precursors.

Nearly 200 different triterpene skeletons are known from natural sources or enzymatic reactions that are structurally consistent with being cyclisation product of squalene, oxidosqualene, or bis-oxidosqualene. Most triterpenoids are 6-6-6-5 tetracycles, 6-6-6-6-5 pentacycles, or 6-6-6-6-6 pentacycles, but acyclic, monocyclic, bicyclic, tricyclic, and hexacyclic triterpenoids have also been isolated from natural sources. An unusually complex and flexible reaction mechanism generates these ring systems. These major groups are again subdivided into subgroups according to structural diversity, like in 6-6-6-5 pentacyclic triterpenes having groups- lupane, betulane, and hopane etc, in 6-6-6-6-6 pentacycles- ursane, oleanane, α and β amyrin etc. Principal pathway of synthesis of terpenoids is as follows :-
In case of triterpene series (C30), two molecules of farnesyl pyrophosphate (C15) are joined in a head-to-head condensation to produce squalene which involves a complex series of cationic rearrangements to accomplish the chemically difficult chore of joining the C-1 carbon of two farnesyl residues and process is catalyzed by squalene synthase, a prenyltransferase. Squalene is usually oxidized to form the 2, 3-epoxide (Oxidosqualene) a precursor of most 3 β-OH-triterpenoids, although squalene cyclization followed by oxidation at C-3 is also plausible. Now enzymatic cyclization of squalene/oxidosqualene gives rise series of tetra, penta and hexacyclic triterpenes, out of them 6-6-6-5-tetracycles, 6-6-6-6-5-pentacycles, and 6-6-6-6-6-pentacycles are most common. As a general mechanism of cyclization, all-trans squalene or oxidosqualene is activated by cationic attack. A cascade of cation-olefin cyclizations then generates a cyclic carbocation, which can rearrange and cyclize further. Antiperiplanar shifts terminated by proton loss then yield a neutral species. After initial cyclization, ring expansion and/or further annulation can generate a modified ring system. The most common triterpene alcohols in plants, such as Lupeol and β-amyrin, originate from the dammarenyl cation, after D-ring expansion via C16 migration followed by 18 β E-ring cyclization and, sometimes, further E-ring expansion. The lupyl cation is generated from the dammarenyl cation by D-ring expansion (C16 migration) to form bachharenyl cation followed by E-ring closure to the β-face of C18. Cationic intermediate lupyl cation has a trans- D, E ring junction. Direct deprotonation without rearrangement provides Lupeol.
Figure 3: Biosynthesis of lupeol 1
1.4. Previous work on Lupeol

Since the structural variations may enhance the biological activities or can introduce desired activity with the help of desired pharmacophores. The two most frequent sites of chemical modification attended in Lupeol for semi-synthetic work are ring-A and isopropenyl side chain.

1.4.1. Modification at ring A

Reduction, esterification, oxidation, dehydrogenation skeletal rearrangements, ring expansion, ring opening etc are the most frequent chemical modifications reported on ring A of Lupeol. These are being discussed here briefly.

Lupeol has been esterified into Lupeol linoleate,\textsuperscript{29} Lupeol succinate and Lupeol succinate sodium.\textsuperscript{90} Glochidon a naturally occurring triterpene was prepared from Lupeol through oxidative-dehydrogenation by using DDQ was subjected to epoxidation followed by LAH reduction and Jone’s oxidation of dihydroxy mixture giving 1β, 3β-dihydroxy Lupeol.\textsuperscript{91} Using a different method for glochidone formation, a more simplified method of oxidative dehydrogenation of lupenone into glochidone has been also reported.

Dehydrogenation of Lupenone is reported by treating them with benzeneselenenic anhydride to give corresponding unsaturated ketones in good yield. However, reaction with excess of benzeneselenenic anhydride for longer time gave an additional product, ring A contracted diketone.\textsuperscript{92} Dehydration of Lupeol has also been studied with POCl\textsubscript{3} in pyridine and by solvolysis of their tosylate, giving Wagner-Meerwein rearrangement products. Dehydrogenation of Lupeol by POCl\textsubscript{3} in pyridine gave A-nor-\Delta\textsuperscript{5} skeleton. While dehydrogenation through solvolysis of Lupeol tosylate by refluxing it with fused NaOAc in dry AcOH gave a mixture of A-nor-\Delta\textsuperscript{3(5)} and \Delta\textsuperscript{5} products.\textsuperscript{93} Ring-A of Lupeol was also dehydrogenated by Alkali fusion to give mixture of 5 and 6 member keto derivatives with 3α-Lupeol.\textsuperscript{94} Ketene dithioacetalization has been studied on ring-A of lupenone. Activated C-2 centre in ring-A of lupenone gave α-oxoketene-di-thioacetal derivative, which was subsequently subjected to ring closure reactions with guanidine hydrochloride and hydrazine hydrate furnishing respective pyrimidine and pyrazole heterocycles condensed with ring A of lupenone.\textsuperscript{95} However, hypervalent iodine oxidation of lupenone with Koser reagent gave various oxidized products without any regio-specificity.\textsuperscript{95} A skeletal rearrangement of ring-A in lupenone was observed when it was treated with powdered KOH in dry DMSO at room temperature, giving an unusual oxidative skeletal rearranged product C-2-nor-1β-hydroxy-2-
oxa-lupenone in excellent yield. This was further treated with MeI giving a ring-A ruptured product, NaBH₄ reduction of which led re-cyclization affording a stereo-isometric mixture of 1-hydroxy-2-oxa-lupan-20(29)-ene.⁹⁶ Lupenone when subjected to Schmidt reaction gave corresponding lactam with ring-A expansion and when treated with excess of N₃H and BF₃-Et₂O in benzene or chloroform, it afforded 3-cyano-4-azido-3,4-seco-lupenone. Beckmann rearrangement on Lupenoneoxime by treating it with POCl₃ or p-TsCl in pyridine at 30-35°C gave a mixture of 3,4-seco nitrile and lactam, while the Beckmann rearrangement using same catalysts in refluxing conditions afforded exclusively 3,4-seco nitrile. Lactam could be converted into 3,4-seco nitrile when refluxed in pyridine with POCl₃.⁹⁷ 3, 4-seco nitrile gave corresponding amine when subjected to reduction with LAH in THF.

Two lupenone units were oxidatively dimerized, attached to each other through ethereal linkage when it was subjected to Willgerodt – Kindler reaction reported previously by our group.⁹⁸

To study the photochemical reactions, dihydrolupenone was irradiated in n-hexane under argon atmosphere using a high pressure mercury lamp, giving a complex mixture and the constituents were identified as a mixture of hydrocarbon, aldehyde, ketone, lactone and acids.⁹⁹

1.4.2. Modification at isopropenyl side chain

Oxidation of isopropenyl side chain with m-CPBA in different conditions is a well studied reaction of Lupeol. Lupeol was oxidized into naturally occurring triterpenoids, 3β-acetoxy-30-nor lupan-20-one, 3β-acetoxy-30-hydroxy-lup-20(29)-ene and 3β-acetoxy-20-hydroxy lupane-29-al when it was treated with m-CPBA in moist chloroform at 0°C. A reinvestigation of oxidation of Lupeol with m-CPBA is also reported with the formation of lup-20-nor-ketone-3β-ol, Lup-20(29)-ene-3β, 30-diol and lup-20-al-3β-ol.¹⁰¹ On the other hand, oxidation of Lupeol by m-CPBA in dry chloroform in refluxing condition afforded 3β-acetoxy-30-hydroxy lup-20 (29)ene.¹⁰² Lupeol acetate was treated with SeO₂ containing H₂O₂ in t-BuOH, which was treated with diazomethane followed by acetylation giving three components, 30-carbomethoxy lupenyl acetate, 30-carbomethoxy lupan 3β, 29diyl acetate and 29-carbomethoxy lupan 3β-yl- acetate.¹⁰³

Lupeol has also been used as a scaffold for the synthesis of Lupeol based libraries using solid phase technique. In this case Lupeol was anchored to a solid support (Rink
amide/Siebermide) through aliphatic dicarboxylic acid moieties which in turn served as a site for introducing diversity. The resulting polymer linked 3β-O (resin-alkanoyl)-lup-20(29)-ene was used to generate key intermediates 3β-O (resin-alkanoyl)-30-bromo-lup-20 (29) ene and 3β-O (resin-alkanoyl)-30-amino-lup-20 (29)-ene for the generation of libraries based on disubstituted Lupeol derivatives. C-30 bromo and C-30 amino functionality were used for further diversification on this molecule giving 96 member libraries for in-vitro antimalarial screening. An acid catalyzed rearrangement was observed in isopropenyl side chain and ring E of Lupeol when it was treated with HBr in Ac₂O-AcOH-benzene at RT for 10 days giving 3β-acetoxy-lup-18-ene and the tetrahydro derivative. Former readily isomerizes into C13-18-ene product. Isomerization of Lupeol skeleton into dammarane and baccharane derivatives is also reported.

1.5. Present work

1.5.1. Basis of Work

Research work is aimed for design and synthesis of pseudo hybrid natural products by chemical modification from which new chemical entities can be made which can be of immense value to medicinal chemists in the discovery of new activity /enhance the existing antimalarial activity. Since the structural variations may enhance the biological activities or can introduce desired activity with the help of desired pharmacophores, more emphasis is now being laid on the development of semisynthetic derivatives which has improved activity or a new biological activity. The reported antimalarial activity and bulk availability of Lupeol makes it a good candidate for improving the activity. On inspection of Lupeol structure it is evident that two possible sites for chemical modification present in Lupeol are ring-A, and isopropenyl side chain. Modification on ring-A, ring-A expansion and cleavage followed by introduction of biologically active pharmacophores and modification at isopropenyl side chain is targeted as represented in figure 1.

![Figure 4: Different loci for modification](image-url)
In present study, it was proposed to synthesize following prototype-

![Chemical Structures](image)

This chapter deals with the chemical modifications of Lupeol and the biological screening of the synthesized compound.

**1.5.2. Isolation of Lupeol**

Lupeol was isolated by extracting stem bark of *Crataeva nurvala* with 95% ethanol. Extract on purification by repeated crystallization gave pure Lupeol along with small amount of lupenone.

Lupeol showed molecular ion peak (M)$^+$ at m/z 426 in its EI mass spectrum. In $^1$H-NMR spectrum (Fig. 6), a pair of two singlets at δ 4.68 and δ 4.56 along with a singlet for 3 protons at δ 1.68 is indicating for the presence of isoprenyl side chain (characteristic of isoprenyl unit) present in molecule. A multiplet for one proton at δ 3.23 for H-3 proton attached to OH, and six singlets for the six methyls of three protons each were present at δ 1.02, 0.96, 0.94, 0.82, 0.78 and 0.76 in Lupeol. Isopropenyl double bond carbons were again
confirmed by signals at $\delta$ 150.78 and $\delta$ 109.28 in $^{13}$C-NMR spectra of this compound. All the spectral details were in accordance with the spectroscopic data available in literature.\cite{108}

**Figure 6:** $^1$H-NMR spectra of 1

### 1.5.3. Chemical modifications

#### 1.5.3.1. Chemical modification at C-30

Allylic methyl group (C-30) in Lupeol, was selectively oxidized by selenium dioxide, in dioxane to give corresponding aldehyde 2 identified by the characteristic peak at $\delta$ 9.50 in $^1$H-NMR spectrum. Lupeol with SeO$_2$ in moist dioxane also gave the same oxidized product with improved yield. Lupeol Aldehyde 2 was also synthesized by using microwave in few seconds with SeO$_2$ (Scheme-1) (Fig 7 & 8).

![Scheme-1](image-url)
Formation of 2 generated an additional functional group (aldehyde) in Lupeol which can be used to build different heterocycles on this bi-functional three carbon unit.
1.5.3.2. Chemical modification at bi-functional three carbon unit (C 20-29 double bond and C 30 aldehyde):

In order to diversify the Lupeol at this end, synthesis of 3-substituted quinoline was carried out by Skraup’s synthesis. Lupeol aldehyde 2 on reaction with substituted aniline in freshly distilled toluene, and conc. H$_2$SO$_4$ as a catalyst gave product 3a-d (Scheme-2).

![Scheme-2](image)

The structure of 3-substituted quinoline 3 was confirmed by the presence of the peaks in the aromatic region (δ 7.3- 8.9) of quinoline nucleus in the $^1$H-NMR spectrum and absence of C 20-29 double bond peak at δ 6.28 and δ 5.90, and aldehyde at δ 9.50 originally present in Lupeol aldehyde 2. HRMS spectra also confirmed that the molecular formula of 3d was C$_{30}$H$_{50}$ClNO. Structure of 3a-d was also confirmed by $^1$H-$^1$H COSY spectra. The reaction is functional group dependent. When the same reaction was carried out with disubstituted anilines or p-anisidine products were not formed, whereas when the amount of conc. H$_2$SO$_4$ used in the reaction was increased a rearranged product was formed in this reaction which was assigned the structure 4. Structure of 4 was confirmed by the decrease of 18 units in mass spectrum from the mass of 3. In $^1$H-NMR spectrum of 4 peak at δ 3.2 for H-3 proton originally present in 2 & 3 was also disappeared (Scheme -2).
In the case of reaction of Lupeol aldehyde with \( o \)-fluoro aniline both the product were obtained \( 5a \) & \( 5b \) which were purified by column chromatography. (Scheme-3) (Fig 9 & 10)

![Scheme-3](image)

Further oxidation of \( 3d \) & \( 5a \) with PCC, gave quantitative yield of \( 6a \) & \( 6b \). Structure of \( 6a \) & \( 6b \) was confirmed by the decrease of 2 units in the FAB mass spectrum and appearance of a peak of carbonyl group at 1640 cm\(^{-1}\) in IR spectrum. A peak at \( \delta \) 3.20 (CH-OH) present in \( 3d \) also disappeared in \( ^1H \)-NMR spectrum. Compound \( 6b \) on treatment with hydroxylamine hydrochloride in pyridine gave oxime \( 7 \) in a good yield (Fig 11), which on reduction with LAH in THF gave corresponding amine \( 8 \). Formation of \( 8 \) was confirmed by (M+H)\(^+\) ion peak at 531 in FAB mass spectrum (Fig 12) (Scheme-4).

![Scheme-4](image)
Figure 9: $^1$H-NMR spectra of 5a

Figure 10: $^1$H-NMR spectra of 5b
Figure 11: Mass spectra of 7

Figure 12: $^1$H-NMR spectra of 8
In another strategy to have other heterocyclic ring around $\alpha$, $\beta$-unsaturated aldehyde, Lupeol aldehyde $2$ was refluxed with hydrazine hydrate in isopropanol gave 4- substituted dihydro pyrazole $9$ whose structure was confirmed by the FAB mass spectrum in which peaks at 454 ($M^+$) appeared. HRMS spectra also confirmed that the molecular formula was $C_{30}H_{30}N_2O$ (Fig 13). In $^1H$-NMR spectrum no additional peak appeared in the double bond region confirmed that double bond was present between the two nitrogen atoms (Fig 14). Similarly pyrimidine and 2,5-dihydro pyrimidine derivatives were synthesized from aldehyde $2$ with amidine hydrochloride and sodium isopropoxide under refluxing condition (Scheme -5).

Scheme-5

Structure of $10a$ was established by appearance of a peak in the down field region at $\delta$ 8.13 in its $^1H$-NMR spectrum (Fig 15). In the $^{13}C$-NMR of compound $10a$ three peaks at $\delta$ 161.7 (C-NH$_2$), $\delta$ 131.8 (Ar-C) and $\delta$ 157.5 (Ar-CH) also confirmed the formation of pyrimidine ring (Fig 16). HRMS also confirmed the molecular formula of $10a$. In the case of benzamidine and 4-amido morpholine derived products, dehydrogenation did not occur and dihydro pyrimidine derivative were obtained whose structure were established by $^1H$-NMR spectrum. FAB mass spectra of $11b$ showed (M+H)$^+$ ion peak at 543 & microanalysis showed the molecular formula $C_{37}H_{34}N_2O$ which proved that the predicted structure was correct. In $^1H$-NMR spectrum in addition to peak of benzene ring protons two additional singlets at $\delta$ 6.1 (N-CH=) for one proton and $\delta$ 4.1 (N-CH$_2$) for two protons appeared confirming the structure of $11b$. 
Figure 13: Mass spectra of 9

Figure 14: $^1$H-NMR spectra of 9
Chapter 1

Figure 15: $^1$H-NMR spectra of 10a

Figure 16: $^{13}$C-NMR spectra of 10a
In another variation Lupeol aldehyde \(2\) was subjected to Wittig reaction with (carbethoxy methylene) triphenyl phosphorane in DCM to yield \(12\) (Scheme-6) whose structure was confirmed by the \(^1\)H-NMR and \(^1\)H-\(^1\)H COSY. In the \(^1\)H-NMR spectrum a quartet present at \(\delta\) 4.2 for two hydrogen (OCH\(_2\)) showed a correlation with a peak at \(\delta\) 1.3 (OCH\(_2\)CH\(_3\)) in \(^1\)H-\(^1\)H COSY spectrum. Two additional doublet appeared at \(\delta\) 7.29 and \(\delta\) 6.0 \((J = 15.8\text{ Hz})\) each for one proton coupled to each other confirmed that double bond formed was \textit{trans} (fig 17). This versatile synthone can be utilized for further reactions.

**Figure 17:** \(^1\)H-NMR spectra of \(12\)
1.5.3.3 Chemical modification at C 20-29 double bond:
An introduction of new functional group (acetyl) was carried out on Lupeol molecule through cleavage of double bond with ozone and a product 13 was formed (Scheme-7).

Structure of 13 was confirmed with the increase of 2 units in ESMS mass spectrum a (M+H)+ ion peak at m/z 429, also a peak at δ 213 appear in 13C-NMR spectrum confirmed that carbonyl group was present in the molecule. HRMS also confirmed that the molecular formula was C29H48O2.

1.5.3.4. Chemical Modification at Ring-A
Diversification of ring-A of Lupeol 1 was initiated with the oxidation of –OH group with PCC to give quantitative yield of lupenone 14 (Scheme-8). This is also a naturally occurring component of stem-bark of Crataeva nurvala, which when treated with hydroxylamine hydrochloride in pyridine gave lupenoneoxime 15 in a good yield, both the product 14 and 15 are in accordance with the spectroscopic data and literature available.97 Lupenone oxime 15 was subjected to reduction with LAH in THF to give corresponding amine 16 in good yield. Formation of 16 was confirmed by the presence of (M+H)+ ion peak at 426 in FAB mass spectrum with an additional peak at 409 (M-NH2). In IR spectrum a peak at 1594 cm⁻¹ disappeared (C=N-OH) which was present in 15 thus confirming the structure of 16.

Ring-A of Lupeol skeleton was made to cleave as well as ring-enlargement from alicyclic 6 membered to 7-membered system through Beckman rearrangement on lupenoneoxime 15, provided a number of chances for diversification in this molecule. Oxime 15 was treated with POCl₃ in pyridine or CDI and allyl bromide in acetonitrile, gave two products, 3,4-seco nitrile 17 (ring-A opening) and a seven membered lactam 18 (ring-A enlarged product). At room temperature it afforded the mixture of products 17 and 18, but when refluxed it yielded exclusively only 3, 4-seco nitrile 17 (Scheme-8). 97 Both the products 17 and 18 were in accordance with the spectroscopic data available.97
In the sequences of diversification in Lupeol skeleton, lactam 18 was reduced to corresponding cyclic amine 19 by using LAH in THF. Overall this was the conversion of 6-membered alicyclic ring-A of Lupeol into 7-membered heterocyclic ring with nitrogen (Scheme-8). Reduced product 19 displayed (M+H)⁺ ion peak at m/z 426 in FAB mass, which was 14 mass units less then the amide 18, did not show anymore IR absorption in the
amide region of IR spectrum, indicating the successful reduction of lactam functionality of $18$ into corresponding cyclic amine $19$. Appearance of N-CH$_2$ signal as a triplet for two protons at $\delta$ 2.77 in its $^1$H-NMR spectrum satisfactory explains the structure of $19$. 

Alkaline hydrolysis of 3, 4-seco nitrile $17$ facilitated the way for synthesis of 3, 4-seco-acid, a naturally occurring triterpene, known as canaric acid$^{109} 20$ (Scheme-8). In FAB mass spectrum of the expected product $20$ ion peak at m/z 441 (M+H)$^+$ is in accordance for the molecular formula C$_{30}$H$_{48}$O$_2$. After hydrolysis IR absorption for cyano group at 2238 cm$^{-1}$ disappeared with the appearance of additional absorption for carbonyl group at 1707 cm$^{-1}$, supporting the conversion of nitrile functionality of $17$ to carboxylic acid $20$. In this way a convenient four-step for conversion of Lupeol $1$ into canaric acid $20$, a naturally occurring 3,4-seco-triterpene was carried out.

Cyclic amine $19$ was N-alkylated by isoprene unit containing halide chains such as prenyl, geranyl and farnesyl etc. Synthesis of $21a$ and $21b$ was carried out when refluxed with alkyl halides in presence of Na$_2$CO$_3$, in dry acetone (Scheme-9). Where as synthesis of $21c$ and $21d$ was achieved when $19$ was treated with sodium hydride and alkyl halide in THF. The structure of $21a-d$ was established by $^1$H-NMR spectrum in which in addition to usual peak of cyclic amine a peak in the region of double bond at $\delta$ 5.1-5.7 also appeared. 

In $^1$H-NMR spectrum of $21c$ besides the usual peaks characteristic multiplets at $\delta$ 5.4 and $\delta$ 5.10 were present for the protons attached to the double bond carbons of the geranyl chain. In the $^1$H-NMR spectra of $21d$ the protons for the N-CH$_2$-CH= double bond was present as a triplet at $\delta$ 5.3 and that for CH$_2$-CH= at $\delta$ 5.10 respectively. Similarly the structures of $21a-b$ were also assigned.

Coupling reaction of cyclic amine $19$ with different cinnamic acid derivatives in the presence of DCC as coupling reagent and DMAP as catalyst gave amides $22a-f$. Structure of these amide were confirmed by $^1$H-NMR spectrum in which two doublets at $\delta$ 7.4 and $\delta$ 7.7 were present for the Ar-CH= and CO-CH= respectively. These two proton were mutually trans coupled ($J$ = 15.5 Hz ). In addition to this aromatic protons were also present in between $\delta$ 7.3- 7.5 which confirms the structure of $22$ (Fig 18). Cyclic amine $19$ on coupling reaction with quinaldic acid gave $23$. IR spectrum of $23$ showed absorption at 1647 cm$^{-1}$ could be assigned for amide (-CO-NH-) grouping as expected for the molecule and additional peaks in aromatic region at $\delta$ 8.21-7.55 in $^1$H-NMR spectrum (Fig 19).
Formylation and acetylation reactions were also carried out on 7-membered cyclic amine 19 when refluxed with formic acid (95%) in acetic anhydride or acetic anhydride in pyridine to give corresponding formamide 24 and acetamide 25 respectively. In FAB mass spectrum of the expected product 24, ion peak at m/z 453 (M)+ was in accordance for the molecular formula C_{31}H_{31}NO. Formation of aldehyde was confirmed by a peak at δ 8.4 in \(^1\)H-NMR spectrum for the -CHO protons.

FAB mass spectrum of 25, showed increase of 42 units in accordance for the molecular formula C_{32}H_{33}NO. Formation of 25 was confirmed by IR spectrum in which a CO stretching band at 1640 cm\(^{-1}\) (N-C=O) appeared.

\[
\begin{align*}
19 & \quad \text{Reflux, Ac}_{2}O, \text{Py} \\
24, R = H & \quad 25, R = CH_{3}
\end{align*}
\]

\[
\begin{align*}
\text{HCOOH, Ac}_{2}O, & \quad \text{Ac}_{2}O/\text{Py, r.t.} \\
\text{RX, K}_{2}\text{CO}_{3}, & \quad \text{Acetone Or} \\
\text{RX, NaH, THF, reflux} & \quad \text{HOBT, DCC, Quinaldic acid} \\
\text{DCM, r.t.} & \quad \text{Acids, DCM, r.t.}
\end{align*}
\]

\[
\begin{align*}
21\text{a}, R = \text{-o-dichloro phenyl} & \\
21\text{b}, R = \text{-p-bromo phenyl} \\
21\text{c}, R = \text{-p-methoxy phenyl} \\
21\text{d}, R = \text{-m-p-dimethoxy phenyl} \\
21\text{e}, R = \text{-m-methoxy phenyl} \\
21\text{f}, R = \text{-m-p-dichloro phenyl}
\end{align*}
\]

Scheme-9
Canaric-acid 20 was subjected to amide formation by different amines. This was achieved when 20 was stirred with HOBT, DCC in DCM, and different amines (piperazine, ethylene...
diamine, and 1,3-diamino propane), gave corresponding amides 26a, 26b, and 26c (Scheme -10). The structure of these amides was confirmed by the peak of N-CH$_2$ at δ 3.3 in $^1$H-NMR spectrum. Formation of 26a-c was confirmed by IR spectrum in which a CO stretching band at 1640 cm$^{-1}$ (N-C=O) appeared. These dimeric amides 26a-c were further reduced with LAH in THF to give corresponding amines 27a-c (Scheme-10). Formation of 27a-c was confirmed by IR spectrum in which a CO stretching band at 1640 cm$^{-1}$ (N-C=O) was missing and decrease of 28 units in the FAB mass spectrums also confirmed the structure.

In another reaction unsaturated sugar D-glucal triacetate and D-galactal triacetate was grafted at 3 position of Lupeol 1 by Ferrier’s rearrangement. D-glucal triacetate was treated with Lupeol in presence of iodine, as catalyst, to give corresponding unsaturated sugar derivative as a α isomer$^{110}$ (major) 28a with unreacted lupeol which could not be purified by column chromatography. This reaction mixture was purified by chemical method and was
treated with acetic anhydride in pyridine so as to acetylate the remaining lupeol which on chromatography yielded compound 28a & 29. Similarly, D-galactal triacetate gave compound 28b. Compounds 28a and 28b were de-acetylated with methanolic ammonia to give 30a and 30b, which in turn were further converted to 31 by allylic oxidation in the presence of magnese dioxide in DCM (Scheme-11).
Figure 20: $^1H-^1H$ COSY spectra of 28 b

Figure 21: $^1H-^1H$ COSY spectra of 31
Formation of 28 was confirmed with an increase of molecular weight by 212 units, m/z 661 (M+Na)^+ in its FAB mass spectrum. Appearance of two additional acetate group as a singlet at δ 2.08 for 6 protons and presence of unsaturated protons as doublets at δ 5.85 (J = 10.5 Hz) for H3' and δ 5.74 (J = 10.2 Hz) for H2' for one proton each, confirmed the structure 30a and 30b. However, for compound 30a and 30b showed (M+H)^+ ion peak at m/z 555 in its FAB mass spectrum, there were no signals of acetyl groups in 1H-NMR. Both 30a and 30b on allylic oxidation gave the same product 31, formation of which was confirmed with a decrease of molecular weight by 2 units and in 1H-NMR signal of 4' proton also disappeared. 1H-1H COSY of both the compounds 28b and 31 also confirmed their structures (Fig 20 and 21).