For thousands of years medicine and natural products (NPs) have been closely linked through the use of traditional medicines and natural poisons. Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines such as aspirin (1), digitoxin (2), morphine (3), quinine (4), and pilocarpine (5).

The discovery of antibacterial filtrate “penicillin” by Fleming in 1928, re-isolation and clinical studies by Chain, Florey, and co-workers in the early 1940s, and commercialization of synthetic penicillins revolutionized drug discovery research. Following the success of penicillin, drug companies and research groups soon assembled large microorganism culture collections in order to discover new antibiotics. The output from the early years of this antibiotic research was prolific and included examples such as streptomycin (6), chloramphenicol (7), chlortetracycline (8), cephalosporin C (9), erythromycin (10), and vancomycin (11). All of these compounds, or derivatives thereof, are still in use as drugs today.

One of the next breakthroughs in drug discovery was the use of mechanism-based screening for bioassay-guided fractionation. Through continual improvement of screening formats, reagent production, robotics, and data management, mechanism-based screening has since become the mainstay of high-throughput screening (HTS). Some of the first compounds identified in the early 1970s using mechanism-based screening methods included the β-lactamase inhibitor clavulanic acid (12) from Streptomyces clavuligerus and the HMG-CoA reductase inhibitor mevastatin (13) (then named ML-236B) from Penicillium citrinum. Mevastatin (13) (then also named compactin) was also reported as an antifungal agent from P. brevicompactum. A mixture of clavulanic acid (12) and amoxicillin (14) (the combination
Summary

is called Augmentin) is still being used today as a front line antibiotic, while mevastatin (13) and lovastatin (15) were the lead compounds for a series of antilipidemic drugs collectively known as the “statins” (Figure 1).

![Fig. 1](image-url)
Despite competition from other drug discovery methods, NPs are still providing their fair share of new clinical candidates and drugs. This was demonstrated recently by Newman, Cragg, and Snader, who analyzed the number of NP-derived drugs present in the total drug launches from 1981 to 2002. They concluded that NPs were still a significant source of new drugs, especially in the anticancer and antihypertensive therapeutic areas. In another study,
Proudfoot reported that 8 out of 29 small molecule drugs launched in 2000 were derived from NPs or hormones and concluded that HTS did not have a significant impact on the derivation of these drugs.

NP-derived drugs are well represented in the top 35 worldwide selling ethical drug sales of 2000, 2001, and 2002 (Figure 2). The percentage of NP-derived drugs was 40% in 2000 and remained approximately constant at 24% in 2001 and 26% in 2002. Therefore, in addition to being a proven and important source of drug leads, NP-derived drugs also contribute significantly to the profitability of many companies.

These factors, as well as an inadequate number of lead compounds in many therapeutic areas and the unique chemical space occupied by NPs, have led to a renewed interest in NP research. However, this renewed interest can be sustained only if NP research can continue to be competitive with other drug discovery techniques. Key factors to remaining competitive include continual improvements in the speed of dereplication, isolation, structure elucidation, and compound supply processes and prudent selection of drug targets for the screening of NP libraries. The present thesis which is divided into three chapters incorporates studies on phytochemical investigation of two important medicinal plants viz-a-viz Delphinium denudatum and Achillea millefolium for the development of drug leads which in turn will contribute very significantly to the profitability of many pharma companies.

**Chapter I** [General introduction] of the thesis deals with the introduction of the Natural products. Natural product research continues to provide significant value in the discovery of novel chemical structures and bioactive lead molecules for clinical development. The descriptions of new compounds published in the past two years that have been cited above indicate that there is not a lack of new chemical diversity in this field. Technological
advances summarized above which can accelerate lead identification and natural product structural elucidation, as well as scale-up and manufacture of final drug products, are encouraging. The large number of compounds derived from natural product sources that are currently under-going evaluation in clinical trials is another positive indicator that natural product discovery provides good value for human medicine. Likewise, the increase in unmet medical needs arising from both a better understanding of disease via the human genome project, as well as from the development of resistance among many of the disease pathogens that historically have been controlled (e.g., S. aureus, tuberculosis, malaria, etc.) provides ample opportunity to rationalize drug discovery from natural products within the pharmaceutical industry.

Realistically, natural product discovery programs require a bit more patience and perseverance for the identification of good lead compounds than do programs strictly limited to synthetic chemicals. The quality of leads arising from natural product discovery is better and often more biologically friendly, due to their co-evolution with the target sites in biological systems. However, the speed at which leads can be generated and advanced is slower than purely synthetic discovery approaches. As was pointed out in previous sections, it is unlikely that the major global pharmaceutical companies will return to natural product discovery programs if they’ve already abandoned this avenue of research. This is due to the limitations listed above, and well as a perception that this research is not cutting-edge. A few companies (e.g., Novartis, Bayer, Wyeth) will continue to conduct natural product research and depend on this type of research to generate development candidates, particularly in oncology and infectious diseases. New virulence-related targets might capture the attention of more drug discovery scientists. Also, testing existing antibiotics for other therapeutic interventions might become more prevalent. However, even at those companies where natural product discovery perseveres, there will be continued pressure to reduce the time and cost of such research, or even eliminate it entirely.

The situation, as it stands in the pharmaceutical industry at the present time, offers to biotechnology and small pharmaceutical companies the opportunity to utilize natural product discovery and succeed at scales that are impossible for the major pharmaceutical companies to operate. New technologies for more rapid identification of bioactive molecules and structural elucidation of novel structures will continue to be leveraged to improve the natural product discovery process. As a result, look for new therapeutic discoveries to arise from
these smaller companies and unmet medical needs to be addressed with natural product derived lead candidates

Chapter II [Phytochemical Investigation of *Delphinium denudatum*] deals with:

a) Cytotoxicity of Extracts of *Delphinium denudatum* Wall: Cytotoxicity of different plant extracts were evaluated using Sulpharhodamine B (SRB) assay. All the samples were screened at 50µg/ml concentration. It was found that most of the samples showed selective cytotoxicity towards different cancer cell lines screened in the present study. Sample DDRC is showing selective cytotoxicity against leukemia (THP-1) and ovarian cancer cell line (OVCAR-5) showing 56% and 99% inhibition respectively. Sample DDRM is again showing selective cytotoxicity against breast cancer cell line (MCF-7) with percentage inhibition of 71%. Sample DDAC is active against leukemia (THP-1), breast cancer cell line (MCF-7) and ovarian cancer cell line (OVCAR-5) showing 56%, 54% and 64% growth inhibition respectively.

b) Antioxidant Activity of Extracts of *Delphinium denudatum*: All the extracts showed a dose dependent scavenging effect on DPPH and ABTS radicals. The chloroform extract of root part of the plant (DDRC) showed the highest DPPH scavenging activity (86.56%) which was higher than the standard ascorbic Acid (82.77%) at the similar dose. On the other hand chloroform extract of floral part of the plant (DDFC) showed the highest scavenging effect (81.21%) on ABTS which was very much comparable to the standard Ascorbic Acid (82.77%) at the similar concentration.

c) Isolation of Chemical Constituents: The air dried aerial and root parts of *Delphinium denudatum* were finely powdered and extracted with petroleum ether, chloroform and methanol (cold). Work up of the methanolic and chloroform extract and separation through chromatographic methods involving repeated column chromatography led to the isolation of seven pure compounds labeled as DD-I, DD-II, DD-III, DD-IV and DD-V, DD-VI and DD-VII. The isolates were identified on the basis of comparison of NMR data ($^1$H and $^{13}$CNMR) with that reported in Literature.

d) LC-MS Profiling of *Delphinium denudatum*: The analysis was separately carried out in case of chloroform as well as methanolic extracts of floral and stem part of the plant. A total of 10 and 8 alkaloids were detected in aerial (DDAC) and floral (DDFC) parts of the chloroform extract of the plant respectively (table) and a total of 10 alkaloids each were detected in methanolic extracts of aerial (DDAM) and
floral (DDFM) parts of the plant. Phytochemical analysis of the alkaloids was carried out by LC-ESI-MS using both positive and negative ion mode. The identification of alkaloids was arrived at by using the MS fragmentation pattern as well as by comparing the molecular ion peaks along with MS fragmentation pattern with those of the literature.

e) Unani Formulations of Delphinium denudatum (Jadwar)

Two unani formulations of Delphinium denudatum (Jadwar) namely Habbe Jadwar and Khameera Gaozaban Ambry Jadwar Ood Saleeb wala were prepared as follows:

Habbe Jadwar: Made powder of all the ingredients. Mixed oil in powder and grinded with Aqua Rosa and finally made tablets of size of Chana (Cicer arietinum seed).

Khameera Gaozaban Ambry Jadwar Ood Saleeb wala: Soaked all the necessary ingredients in water at night and boiled them in the morning till water remained half. Rubbed and filtered it. Added sugar and Asl (Honey) and made Qiwm (Basic Solution of Particular consistency). Lastly added Amber, Verq Nuqra (Silver leaf), Verq Tila (Gold Leaf), powder of Jadwar (Delphinium denudatum root) and Powder of Ood Saleeb (Paonea officinalis root). Triturated it, as it became white.

Chapter III [Phytochemical Investigation of Achillea millefolium] deals with:

a) Cytotoxicity of Extracts of Achillea millefolium: The cytotoxic effects of the extracts prepared from all the three plant parts separately (root, aerial and flower) of Achillea millefolium were evaluated against THP, PC-3, MCF-7 and OVCAR-5 cell lines using SRB assay system. The dried plant material of root, aerial and floral parts were separately subjected to extraction with methanol. The methanol extracts of all the three parts were subsequently partitioned yielding chloroform and methanol soluble extracts. Both the chloroform and methanol extracts (AMAM, AMAC, AMFM, AFMC, AMRC and AMRM) were screened for cytotoxicity against a panel of four cell lines at 50 µg/mL concentration. The chloroform extract of flowers exhibited noteworthy tumor cell growth inhibitory activity against Leukemia/THP, Breast/MCF-7 and Ovary/OVCAR-5 cell lines with percentage growth inhibition of 99, 98 and 99% respectively. On the other hand chloroform extract of roots (AMRC) shows selective cytotoxicity against leukemia cell line with 98% growth inhibition.

b) Antioxidant Activity of Axtracts of Achillea millefolium: The antioxidant activity of all the six extracts of root, aerial and floral parts (AMAM, AMAC, AMFM, AMFC, AMRM and AMRC) were evaluated using two different antioxidant assay systems: DPPH and ABTS. )
The chloroform extract of root (AMRC) shows the highest DPPH radical scavenging activity with an IC$_{50}$ of 2.5 µg/mL comparable to that of reference standard ascorbic acid (IC$_{50}$ of 2.5 µg/mL). On the other hand, methanol extract of the aerial part of Achillea millefolium (AMAM) exhibited a dose dependent highest oxidation potential of all the six extracts screened for antioxidant activity using ABTS assay system with an IC$_{50}$ of 2.3 µg/mL. The chloroform extract of root was selected for a detailed phytochemical-pharmacological analysis so that the antioxidant constituents could be isolated and identified. The isolated compounds were screened for antioxidant activity using DPPH assay system. All the isolates showed potent radical scavenging activity with IC$_{50}$ value of 2.7, 2.2, 3.1, 2.2, 2.26 and 6.34 µg/mL for chlorogenic acid, quercetin, apigenin-7-O-glucoside, luteolin-7-O-glucoside, dihydroquercetin and Salvigenin respectively.

c) Isolation of Chemical Constituents: Rigorous phytochemical analysis of chloroform extract of root afforded six compounds viz. chlorogenic acid, quercetin, apigenin-7-O-glucoside, luteolin-7-O-glucoside, salvigenin and dihydroquercetin. The isolates were identified on the basis of comparison of NMR data ($^1$H and $^{13}$CNMR) with that reported in literature.

d) Chemical Composition of the Essential oil: GC-MS analysis of the essential oil led to the identification of 15 constituents accounting for 97.252 % of the total oil composition. The principal components of the essential oil are Camphor (23.48%), 1,8- Cineole (22.88%), Germacrene - D (11.39%), Chrysanthenone (9.86%), Sabinene (7.073%), Borneol (4.34%), (z) - beta - farnesene (3.621) α-pinene (3.062%). The chemical composition of essential oil A.millefolium from Kashmir valley using hydrodistillation technique shows both qualitative as well as quantitative differences with that reported from other parts of the world.

e).Antibacterial Activity: The in vitro antibacterial activity of the essential oil of A.millefolium against the tested Gram-positive and Gram-negative bacteria was qualitatively and quantitatively assessed by the inhibition zones. The results indicate that the essential oil exhibited a broad spectrum and potent inhibitory effect against all tested bacterial strains (S. aureus, B. subtilis, E. coli, P. aeruginosa, K. pneumonia, and S. flexneri). The essential oil showed a better antimicrobial effect against the two Gram-positive bacterial strains S. aureus and B. subtilis with the MIC values of 20.02 µg/mL and 17.57 µg/mL respectively. Gram-negative bacterial strains were a little resistant than their Gram-positive counterparts with the MIC values in the range of 33.90µg/mL to 40.21 µg/mL.
e) Unani Formulations of *Achillea millefolium* (Biranjasif): The most common unani formulation of *Achillea millefolium* called as Arq Biranjasif was prepared.

*Arq Biranjasif:* Soaked all the ingredients in water at night. In the morning obtained Aab Mako Sabz (*Solanum nigrum* Herb juice) and added to drugs soaked in water.