1.1. Natural Products

Nature is an ancient pharmacy that used to be the solitary source of therapeutics for the early eras. Ancient civilizations of both China and India have provided a wealth of knowledge on the use of traditional medicines. At the beginning of the nineteenth century, the era of “modern” drugs began. In the 1800s, the most widely used drug in the world was synthesized by Felix Hoffmann, known as aspirin. In 1805, the first pharmacologically active compound morphine was isolated by Friedrich Serturner, from the opium plant.\(^1,2\) Subsequently, countless active compounds have been separated from natural sources. The World Health Organization (WHO) estimated that 80% of the earth inhabitants mainly depend on traditional medicines for their health care.\(^3\) Medicines, such as anticancer, antihypertensive, and antimigraine have benefited greatly from natural products.\(^1,4\)

1.1.1. Sources of natural products

The anecdote of bioactive natural products started more than 100 years ago. Their usual definition in the widest sense is chemical compounds isolated/derived from the nature \(i.e.\) living organisms such as plants, animals, marines and microorganisms. These compounds may be derived from primary or rather secondary metabolism of this organisms.\(^5\) Chemistry of natural products is related to the isolation, biosynthesis and structure elucidation of new products that led to new biological agents. On account of their chemical diversity and various activities against diseases, they have been playing an important role in pharmaceutical and agricultural research.\(^6\) Approximately half of all new drugs in the time frame reported are of natural origin or designed on the basis of natural product structure\(^7\) and nearly half of the 20 best-selling non protein drugs are related to natural products.\(^8\) The paclitaxel (Taxol), most widely used breast cancer drug have been isolated from the bark of \(Taxus\ brevifolia\) (Pacific Yew).\(^9\) In 1992, FDA approved Taxol for various uses.\(^10\) Arteether, introduced in 2000, as Artemotil is derived from artemisinin which was first isolated from the plant \(Artemisia\ annua\) and are both approved antimalarial drugs.\(^11\) Grandisines A and B, indole alkaloids were isolated from the leaves of \(Elaeocarpus\ grandis\), exhibit binding affinity for the human \(\delta\)-opioid receptor and are potential leads for analgesic agents.\(^12\) Apomorphine is a derivative of morphine isolated from the poppy (\(Papaver somniferum\)), used to treat Parkinson’s disease.\(^13\) Tubocaurarine, used as a muscle relaxant in surgical operations was isolated from \(Chondrodendron\)
**tomentosum.** Beside this, several other plant-derived compounds presently used in clinical trials are; Digitoxin, Podophyllotoxin, Compotehin, Vincristine, Vinblastine, Epipodophyllotoxin, Bruceatin, Flavopiridol etc.\(^{15-17}\)

### 1.1.2. Classes of natural products

Plants produce an enormous variety of natural products with highly diverse structures. The compounds are classified into four different groups according to their biosynthetic origin: flavonoids, alkaloids, terpenoids and coumarins.

Flavonoids are polyphenolic compounds, present ubiquitously throughout the plant kingdom. They embrace a wide range of substances, which possess 2-phenyl-benzyl-\(\gamma\)-pyrone, in their structural framework. Their biosynthesis pathway (part of the phenylpropanoid pathway) begins with the condensation of one \(p\)-coumaroyl-CoA molecule with three molecules of malonyl-CoA to yield chalcone, catalyzed by chalcone synthase (CHS). The next step is isomerization of chalcone to flavanone by chalcone isomerase (CHI).\(^{14}\) From this step onwards, the pathway branches to different flavonoid classes, including aurones, dihydrochalcones, flavanonols (dihydroflavonols), isoflavones, flavones, flavonols, leucoanthocyanidins, anthocyanins and proanthocyanidins.

Alkaloids are defined as heterocyclic nitrogen compounds, biosynthesized from amino acids. Many other substances, however, that do not exactly match this rule are classified as alkaloids, either for historical reasons or due to their bioactivities. With currently more than 12,000 known structures, alkaloids represent one of the biggest group of natural products.\(^{18}\) Due to this large number and the high structural diversity, it is impossible to give a comprehensive summary of all different types of alkaloids.

Terpenoids, also named isoprenoids, are the largest class of natural products in plants and comprise more than 40,000 different structures. They are derived from five-carbon isoprene units, and according to the number of isoprene molecules incorporated, they can be classified into hemiterpenes (C\(_3\)), monoterpenes (C\(_{10}\)), sesquiterpenes (C\(_{15}\)), diterpenes (C\(_{20}\)), triterpenes (C\(_{30}\)), tetraterpenes (C\(_{40}\)), and polyterpenes.\(^{14}\) In plants, terpenoids originate from two different biosynthetic routes\(^{19}\): the cytosolic mevalonic acid (MVA) pathway and the plastid-located desoxyxylulose phosphate (DXP) pathway (also called methylerythritol phosphate or MEP pathway). Both biosynthetic routes yield the activated isoprene units dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP), which are
joined by head-to-tail or tail-to-tail linkage and subsequently can undergo cyclization and other modifications e.g., oxidations or rearrangements. While hemiterpenes, monoterpenes, diterpenes, and tetraterpenes are derived from the DXP pathway, triterpenes, steroids, and certain sesquiterpenes originate from mevalonic acid.

Literature survey reveals that the natural products possess inherent wide range of pharmacological activities such as anticancer, antimicrobial, antioxidant and antiviral activities. Some of the examples have been summarized in Table (1-4).

<p>| Table 1 Biological activities of naturally occurring Flavonoids |
|---------------------|-------------------|----------|
| Name                | Structure         | Source              |
| Anticancer          |                   |                      |
| Baicalin            | <img src="image" alt="Baicalin Structure" /> | <em>Scutellaria baicalensis</em>&lt;sup&gt;20&lt;/sup&gt; |
| Eryvarin            | <img src="image" alt="Eryvarin Structure" /> | <em>Erythrina mildbraedit</em>&lt;sup&gt;21&lt;/sup&gt; |
| Antioxidant         |                   |                      |
| Lanneaflavonol      | <img src="image" alt="Lanneaflavonol Structure" /> | <em>Lannea alata</em>&lt;sup&gt;22&lt;/sup&gt; |</p>
<table>
<thead>
<tr>
<th><strong>Antimicrobial</strong></th>
<th><strong>Antiviral</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin</td>
<td>Leachianone G</td>
</tr>
<tr>
<td><img src="image" alt="Rutin" /></td>
<td><img src="image" alt="Leachianone G" /></td>
</tr>
<tr>
<td><strong>Hedysarum carnosum</strong>&lt;sup&gt;23&lt;/sup&gt;</td>
<td><strong>Morus alba</strong>&lt;sup&gt;25&lt;/sup&gt;</td>
</tr>
<tr>
<td>Papyriflavonol A</td>
<td>Vogelin J</td>
</tr>
<tr>
<td><img src="image" alt="Papyriflavonol A" /></td>
<td><img src="image" alt="Vogelin J" /></td>
</tr>
<tr>
<td><strong>Broussnetia papyrifera</strong>&lt;sup&gt;24&lt;/sup&gt;</td>
<td><strong>Ficus virens</strong>&lt;sup&gt;26&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sophoraisoflavanone A</td>
<td>Echinosophora koreensis**&lt;sup&gt;24&lt;/sup&gt;**</td>
</tr>
<tr>
<td>Table 2 Biological activities of naturally occurring Alkaloids</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Anticancer</strong></td>
<td></td>
</tr>
<tr>
<td>Khasuanine A</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Khasuanine A" /></td>
<td></td>
</tr>
<tr>
<td><em>Melodinus khasianus</em></td>
<td></td>
</tr>
<tr>
<td>Sanguinarine</td>
<td></td>
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<tr>
<td><img src="image" alt="Sanguinarine" /></td>
<td></td>
</tr>
<tr>
<td><em>Sanguinaria canadensis</em></td>
<td></td>
</tr>
<tr>
<td><strong>Antimicrobial</strong></td>
<td></td>
</tr>
<tr>
<td>Sampangine</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Sampangine" /></td>
<td></td>
</tr>
<tr>
<td><em>Cananga odorata</em></td>
<td></td>
</tr>
<tr>
<td>Isocorydine</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Isocorydine" /></td>
<td></td>
</tr>
<tr>
<td><em>Berberis microphylla</em></td>
<td></td>
</tr>
<tr>
<td><strong>Antioxidant</strong></td>
<td></td>
</tr>
<tr>
<td>Vindolicine</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Vindolicine" /></td>
<td></td>
</tr>
<tr>
<td><em>Catharanthus roseus</em></td>
<td></td>
</tr>
</tbody>
</table>
Table 3 Biological activities of naturally occurring Terpenoids

<table>
<thead>
<tr>
<th>Antimicrobial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleananoic acid acetate</td>
</tr>
<tr>
<td>Rediocide C</td>
</tr>
<tr>
<td><strong>Antiviral</strong></td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Biperovskatone</td>
</tr>
<tr>
<td><img src="image" alt="Biperovskatone" /></td>
</tr>
<tr>
<td><em>Perovskia atriplicifolia</em>[^37]</td>
</tr>
</tbody>
</table>

[^37]: Reference text
[^38]: Reference text
[^39]: Reference text
[^40]: Reference text
[^41]: Reference text
Table 4: Biological activities of naturally occurring Coumarins

<table>
<thead>
<tr>
<th>Activity</th>
<th>Compound</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticancer</td>
<td>Imperatorin</td>
<td><em>Angelica dahurica</em>&lt;sup&gt;43&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Osthole</td>
<td><em>Ferulago campestris</em>&lt;sup&gt;44&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antimicrobial</td>
<td>Anthogenol</td>
<td><em>Aegle marmelos</em>&lt;sup&gt;45&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Agasyllin</td>
<td><em>Ferulago campestris</em>&lt;sup&gt;46&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antiviral</td>
<td>Inophyllum A</td>
<td><em>Calophyllum inophyllum</em>&lt;sup&gt;47&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The table shows the biological activities of naturally occurring Coumarins, with compounds and their sources specified for each activity.
A large number of naturally occurring novel compounds of therapeutic importance have been isolated to every year. Although it is not possible to cover all the literature related to the isolation of natural products from plant sources, some important, recent and relevant naturally occurring compounds are as follows:

**Flavonoids**

Liu et al.\textsuperscript{50} have isolated three new carboxylated flavonoids, uncinatic acids A-C (1-3), from the herb of \textit{Selaginella uncinata}. In addition, the isolates were tested for their cytotoxicity against A549 and BGC-823 cell lines \textit{in vitro}. 
Zou et al.\textsuperscript{51} and co-workers isolated six new flavonoids, unciflavones A-F (4-9), from medicinal plant \textit{Selaginella uncinata}.

Long et al.\textsuperscript{52} have isolated six new flavonoids, involvenflavones A-F (10-15), from \textit{Selaginella involven}. These compounds also exhibited a potent effect against the injury of human umbilical vein endothelial cell (HUVECs) induced by high concentrations of glucose \textit{in vitro}. 
Zou and co-workers\textsuperscript{53} reported the isolation of six new flavonoids, seladoeflavones A-F (16-21), from the herbs of *Selaginella doederleinii*, along with one known flavonoid (22). In addition, bioassay of the isolates revealed that 20-22 exhibited moderate cytotoxicity against three human cancer cell lines NCI-H460, A549, and K562 *in vitro* with IC\textsubscript{50} values ranging from 8.17 to 18.66 $\mu$M.
Xu et al.\textsuperscript{54} have isolated two new flavonoids, saniculamins A and B (23 and 24), together with three known flavonoid derivatives brosimacutin, 6-flavonol, and eucomol from the whole plants of \textit{Sanicula lamelligera}.

Alkaloids

Sun et al.\textsuperscript{55} reported the isolation of four new dimeric bromopyrrole alkaloids (26-29), including hexazosceptrin (25).

Jin and co-workers\textsuperscript{56} have isolated two new imidazole alkaloids, lepidiline C and D (30 and 31) along with two known imidazole alkaloids (lepidiline A and B), from the root of \textit{Lepidium meyenii}.
Chen et al.\textsuperscript{57} isolated two new alkaloids, nigellisoquinomine (32) and nigellapyrrolidine (33) along with two known alkaloids, agrocybenine and 4,6,6-trimethyl-3,4-epoxypiperidin-2-one, from the seeds of Nigella glandulifera Freyn. Compound 32 exhibited potent protein tyrosine phosphatase 1B (PTP1B) inhibitory activity with an IC\textsubscript{50} value of 3.65±0.08 mM.

Dong and co-workers\textsuperscript{58} have reported the isolation of two new alkaloids, dehydrostenine A (34) and B (35), from the roots of Stemona sessilifolia.

Yang et al.\textsuperscript{59} have isolated four tropane alkaloids, a new compound triuniamine A (38) along with previously reported known compounds darlingine (36), 10-hydroxydarlingine (37) and 2,3-dihydrodarlingine (39) from the stems of Triunia montana.

Coumarins

Aminudin et al.\textsuperscript{60} have reported the isolation four new 4-substituted coumarins, incrassamarin A (40), B (41), C (42) and D (43) with (7S,8S)-7,8-dihydro-5-hydroxy-7,8-dimethyl-4-propyl-2H,6H-benzo[1,2-b;5,4-b']dipyran-2,6-dione (44), friedelin, carpachromene, amentoflavone, epiafzelechin and L-quercitrin from the barks and leaves of Calophyllum incrassatum. Compound (40) displayed cytotoxic activity
against A-549 cell lines with IC\textsubscript{50} 87.71 mg/mL and showed inhibition towards α-glucosidase enzymatic activity with IC\textsubscript{50} 93.25 mM.

Wanga et al.\textsuperscript{61} have been isolated two new dicoumarins, chimsalicifoliusins A (45) and B (46), a new tricoumarin, chimsalicifoliusin C (47), and nine known coumarin from \textit{Chimonanthus salicifolius}. Compounds 1-3 showed modest cytotoxicity against Hela and HL-60 cell lines, with IC\textsubscript{50} values ranging from 14.2 to 29.6 mM, while only chimsalicifoliusin C (47) had the cytotoxicity against PC-3 cell line.

Bashir et al.\textsuperscript{62} reported the isolation of two new sesquiterpene coumarins, fnarthexone (48) and fnarthexol (49), along with three known coumarin derivatives,
conferol, conferone and umbelliferone from the plant *Ferula narthex* Boiss. Conferol was found to be the most potent with IC$_{50}$ value of 11.51, 0.09 mg/mL *in vitro* leishmanicidal activity.

![Chemical structures](image)

*Dastan* and co-workers$^{63}$ isolated a new disesquiterpene and five sesquiterpene coumarins from the roots of *Ferula pseudalliiacea*. Compound 50, 54 and 55 displayed the highest potency against HeLa cells with IC$_{50}$ of 2.2, 6.7, and 4.9 µM, respectively.

![Chemical structures](image)

*Liu et al.$^{64}$* isolated two new hexahydrobenzo[c]phenanthridine alkaloids, ambiguine H (56) and ambiguine I (57) together with six known alkaloids (58-63), from the *Corydais ambigua* var. amurensis leaves. All the compounds except
compound 61 showed the remarkable protective effect on myocardium ischemia-hypoxia cells and compound 59 was the most active compound with cell viability of 49.4, 53.3, 68.2 and 84.5%, which were stronger than that of salvia acid B.

\[ \text{56-62} \]

\[ \begin{align*}
56 & \quad R_1=R_3=R_4=\text{OH}, R_2=\text{OCH}_3; \\
57 & \quad R_1=\text{OCH}_3, R_2=R_3=R_4=\text{O}; \\
58 & \quad R_1=R_2=R_4=\text{OCH}_3, R_3=\text{OAc}; \\
59 & \quad R_1=R_2=R_4=\text{OCH}_3, R_3=\text{OH}; \\
60 & \quad R_1=R_2=\text{OCH}_3, R_3=R_4=\text{OH}; \\
61 & \quad R_1=R_3=\text{OH}, R_2=R_3=\text{OCH}_3; \\
62 & \quad R_1=R_2=\text{OCH}_3; \\
63 & \quad R_3=R_4=\text{OH}
\end{align*} \]

Sakunpak and co-workers\(^\text{65}\) isolated two new monoterpene coumarins, minutin A (\(64\)) and minutin B (\(65\)), from \textit{Micromelum minutum} leaves along with four known coumarins.

\[ \text{64} \]

\[ \text{65} \]

\[ \text{1.2. Green Chemistry} \]

The world-wide synthetic community has been already aware about the development of green chemistry processes, where non-toxic substances can be used and the generation of waste can be avoided. Green synthesis protocols not only provide essential atom-economy, energy savings, waste reduction, and easy workup but also avoid hazardous chemicals.\(^\text{66}\) Greener aspects in molecular design now invariably include the use of bio-renewable raw materials in benign reaction media and recyclable nano-catalysts in atom-economical synthesis as thrust areas.\(^\text{67}\) This may encompass an unconventional reaction activation methodology, such as mechano-chemical mixing, catalysis, solvent less reactions and microwave and ultrasonic irradiation. The object of green chemistry is not only the development of novel methods but is to develop alternative sustainable variants to existing ones. Thus, in order to design new reaction systems satisfying the green chemistry principles, we have carried out the synthesis of bioactive compounds \textit{viz.} hydrazones, pyrazolones...
and acrylonitriles by employing ionic liquids, microwave and silica supported catalysts.

1.2.1. Ionic liquids

Ionic liquids (ILs), are ionic salts that are liquids at ambient or below ambient temperatures have been widely utilized as promising alternatives to hazardous, toxic, volatile and highly flammable organic solvents. In fact, various attractive and unique physicochemical properties of ILs such as extremely low vapor pressures, high salvation interactions with inorganic and organic compounds, excellent thermal and chemical stabilities, good ionic conductivities and broad electrochemical windows make ILs attractive candidates for the replacement of volatile organic compounds. The combination of all these unique properties opens new avenues to an extensive range of applications, including, organic synthesis and catalysis, extraction, inorganic synthesis, nanomaterial synthesis, separation, biocatalysis, pharmaceuticals and polysaccharide dissolution.

Hu et al.\textsuperscript{69} reported condensation of Meldrum’s acid with aromatic aldehydes proceeded efficiently in a reusable ionic liquid, ethylammonium nitrate (EAN), at room temperature in the absence of any catalyst with high yields.

\[
\text{O} \quad \text{O} \quad \text{O} \quad \text{Ar} \quad \text{H} \quad \text{EAN, RT} \quad 0.5-2 \text{ h}
\]

\[
\text{O} \quad \text{O} \quad \text{O} \quad \text{Ar} \quad \text{H}
\]

\[\text{Ar} = \text{C}_6\text{H}_5, \text{p-}\text{Me}_2\text{NC}_6\text{H}_4, \text{p-}\text{MeOC}_6\text{H}_4, \text{p-}\text{OHC}_6\text{H}_4, \text{3,4-(OCH}_2\text{)}\text{C}_6\text{H}_3, \text{p-ClC}_6\text{H}_4, \text{p-NO}_2\text{C}_6\text{H}_4, \text{o-NO}_2\text{C}_6\text{H}_4, \text{2-Furyl, C}_6\text{H}_5\text{CH=CH}
\]

Xu et al.\textsuperscript{70} have introduced, 1-methyl-3-butylimidazolium hydroxide ([bmim]OH) as a novel basic ionic liquid catalyst for the Markovnikov addition of \textit{N}-heterocycles to vinyl esters under mild conditions.

\[
\text{R}_2 \quad \text{N} \quad \text{R}_1 + \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{[bmim]OH} \quad 50^\circ\text{C}, 2-12 \text{ h}
\]

\[
\text{R}_2 \quad \text{N} \quad \text{R}_1 \quad \text{O} \quad \text{R}_3
\]

\[\text{R}_1 = \text{H, CH}_3; \text{R}_2=\text{NO}_2, \text{H, CH}_3; \text{R}_3=\text{CH}_3, \text{CH}_3(\text{CH}_2)_2, (\text{CH}_3)_2\text{CH, CH}_3(\text{CH}_2)_3, \text{CH}_3(\text{CH}_2)_4, \text{Ph}
\]
1.2.2. Microwave assisted synthesis

Among the different aspects of green synthetic methods, synthesis involving microwave irradiation has gained more popularity as a powerful tool for rapid and efficient synthesis due to selective and efficient absorption of microwave (MW) energy by polar molecules.\(^1\) Microwave synthesis has considered as a green technology because it allows solvent-free reactions and low energy consumption compared to traditional methods. The short reaction time and expanded reaction range offered by microwave-assisted organic synthesis are suited to the increased demands in industry. As, there is a requirement in the pharmaceutical industry for a higher number of novel chemical entities to be produced, which requires chemists to employ a number of resources to reduce the time for the production of compounds.\(^2\)

Tu et al.\(^3\) synthesize a series of furo[3′,4′:5,6]pyrido[2,3-\textit{d}]pyrimidine derivatives by three-component reactions between an aldehyde, 2,6-diaminopyrimidine-4(3\textit{H})-one, and tetronic acid/indane-1,3-dione, without using any catalyst in MW.

\[
\begin{align*}
\text{Tu et al.}\; &\;\;\; (72) \\
\text{Tu et al.}\; &\;\;\; (73) \\
\text{Taran et al.}\; &\;\;\; (74)
\end{align*}
\]

R= H, 4-Cl, 4-Br, 4-F, 4-Me, 2-Cl, 2-NO\(_2\)

Taran et al.\(^4\) have reported the use of Cu (I)-species anchored to functionalized chitosan microspheres to obtain 1,4-substituted triazoles at 150 °C in 15 min.

\[
\begin{align*}
\text{Taran et al.}\; &\;\;\; (76) \\
\text{Taran et al.}\; &\;\;\; (77) \\
\text{Taran et al.}\; &\;\;\; (78)
\end{align*}
\]

Singh et al.\(^5\) outlined an efficient one-pot synthesis of substituted pyridines in high yields using a multicomponent reaction of aromatic aldehydes, malononitrile, and thiophenol in ethanol using KF/Alumina in MW at 80 °C.
Heterogeneous supported catalysts have been gained much attention in recent years, as they possess a number of advantages. Immobilization of catalysts on solid support improves the available active site, stability, hygroscopic properties, handling and reusability of catalysts which all factors are important in industry. Therefore, use of supported and recoverable catalysts in organic transformations has economical and environmental benefits. Lewis acid immobilized on solid surface have gained much attention in organic synthesis, e.g. hydroxyapatite supported Lewis acid catalyst has been developed for the transformation of trioses in alcohols, Nb2O5·nH2O has been described as heterogeneous catalyst with water tolerant Lewis acid sites, silica gel supported aluminium chloride has been reported for the solvent-free synthesis of bis-indolylmethanes, polystyrene supported Al(OTf)3 was used for the synthesis of acylals from aldehydes.

Jetti et al. reported silica-bonded N-propyl sulfamic acid (SBNPSA) catalyzed one-pot three component biginelli condensation of different substituted aromatic aldehydes with ethyl acetoacetate and urea/thiourea to the respective 3,4-dihydropyrimidin-2-(1H)-ones and thiones.

Li et al. used silica-supported aluminum chloride for one-pot manich-type reactions of acetophenone with aromatic aldehydes and aromatic amines.
Bigdeli and coworkers\textsuperscript{92} successfully synthesized xanthane derivatives using silica supported perchloric acid (HClO\textsubscript{4}-SiO\textsubscript{2}) as a catalyst.

\begin{align*}
\text{87} & \quad \text{88} & \quad \text{89} & \quad \text{90} \\
R_1= \text{H, 4-CH}_3\text{O, 4-CH}_3, 4-\text{Br, 4-Cl, 4-OH, 4-N(CH}_3\text{_2)}; & R_2= \text{H, 4-CH}_3, 3-\text{NO}_2, 4-\text{Cl, 3-Br, 3-COOH, 4-COOH, 2-Cl, 2-NO}_2
\end{align*}

1.3. Methods of Chemical Analysis

In recent years, immense development in the field of natural products chemistry and synthetic organic chemistry has taken place due to the availability of powerful analytical techniques. The spectral techniques provide substantial information regarding the structure of individual compounds. The spectroscopic techniques like infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (\textsuperscript{1}H NMR and \textsuperscript{13}C NMR) and mass spectrometry (MS) have been used for the structure elucidation and identification of the synthesized and natural compounds. A brief review on spectroscopic techniques like UV, IR, \textsuperscript{1}H NMR, \textsuperscript{13}C NMR and MS has been discussed here in the present study by taking a relevant example.

1.3.1. Ultraviolet Spectroscopy

Ultraviolet spectroscopy has become a major technique for the structure elucidation of natural compounds especially flavonoids.\textsuperscript{93} The UV spectra of flavones in methanol typically exhibit two major absorption peaks in the region 240-400 nm. Band I at around 300-380 nm due to cinnamoyl moiety and Band II at around 240-240 nm due to benzoyl moiety. On increasing oxygenation of the B-ring in flavones, a bathochromic
shift in Band I occurs, while Band II remains unaffected. Further on increasing hydroxylation of the A-ring in flavones produces a bathochromic shift in Band II with a smaller effect on Band I. Isoflavones can easily distinguished from flavones by their UV spectra. The UV spectra of isoflavones typically exhibited an intense Band II adsorption with only a shoulder or low intensity peak representing Band I. The Band II adsorption of isoflavones usually occurs at around 245-270 nm. The Band II of isoflavones is unaffected by the increased hydroxylation of B-ring, however, Band II shifted bathochromically by increasing oxygenation in the A ring. In case of chalcones, the major absorption band (Band I) usually occurs in the range of 340-390 nm.

The structural information obtained from the UV spectrum could be further confirmed by the use of specific shift reagents such as sodium methoxide (NaOMe), sodium acetate (NaOAc), aluminium chloride (AlCl₃) and aluminium chloride/hydrochloric acid (AlCl₃/HCl) sodium acetate/boric acid (NaOAc/H₃BO₃). The addition of these shift reagents separately to an alcoholic solution of the flavonoids lead to a significant shifts in the UV absorption bands and thus provides sufficient information about the orientation of the various hydroxyl groups present in the flavonoid nucleus.

The UV spectra of Isoflavones containing A-ring hydroxyl groups usually show bathochromic shift in the presence of NaOMe for both Band I and Band II. NaOAc, being a weaker base than NaOMe, hence it ionizes the more acidic 7-hydroxyl group in isoflavone causing a bathochromic shift of 6-20 nm in Band II. NaOAc/H₃BO₃ mixture is used for the detection of A-ring ortho-dihydroxyl groups in isoflavones. However, the ortho-dihydroxyl groups in B-ring are not detectable by this shift reagent because the B-ring lacks effective conjugation with the major chromophore. On addition of NaOAc/H₃BO₃, a bathochromic shift by 10-15 nm in Band I is
observed in presence of 6,7-dihydroxyl group in the A-ring of isoflavones. Further in presence of AlCl₃, the ortho-dihydroxyl groups in B-ring are not detectable due to less or no conjugation with the major chromophore, however in absence of C-5 hydrogen bonded hydroxyl group, the ortho-dihydroxyl groups in ring-A can easily detected by the use of AlCl₃. The 6,7- or 7,8-dihydroxyl isoflavones exhibit bathochromic shift for both Band I and Band II using AlCl₃/HCl. The UV spectra of all 5-hydroxyisoflavones undergo a consistent 10-14 nm bathochromic shift in Band II in the presence of AlCl₃/HCl. However, the spectra of isoflavones lacking a free 5-hydroxyl group are unaffected by this reagent.⁹⁴

The UV spectra of chalcones in the presence of NaOMe causes a bathochromic shift of 60-100 nm of Band I for 4-hydroxyl, 2-hydroxyl and 4'-hydroxyl group. There is an increase in the peak intensity along with bathochromic shift in case of 4-hydroxyl group while the other two do not affect the intensity. The ortho-dihydroxyl groups in B-ring of chalcones are readily detected by the 28-36 nm bathochromic shift observed in Band I, while A-ring ortho-dihydroxyl groups show smaller shift in UV spectra of chalcones on addition of NaOAc/H₃BO₃.⁹⁴ The ortho-dihydroxyl groups in B-ring of chalcones can also be detected by a 40-70 nm bathochromic shift of Band I (relative to the Band I position in the AlCl₃/HCl UV spectrum) on the addition of AlCl₃. A-ring ortho-dihydroxyl groups can also be detected by this procedure with smaller shift in UV spectra. Band I in the UV spectra of 2'-hydroxylchalcones usually undergoes a large bathochromic shift of 48-64 nm in the presence of AlCl₃/HCl, however, in the spectra of 2',3',4'-trihydroxylchalcones and its derivatives, the shift is only about 40 nm. Lower wavelength bands also appear to shift bathochromatically, but since these bands are often poorly defined, the shifts are difficult to determine.

1.3.2. Infra-red Spectroscopy
Infrared Spectroscopy is the analysis of interaction of infrared light with a molecule. The IR spectroscopy is based on the absorption of the infrared radiation by molecule which causes an excitation of molecule from a lower to the higher energy levels. It is a simple and reliable technique used for the identification of the natural and synthesized compounds. IR spectrum usually extends from radiation around 4000 cm⁻¹ to 400 cm⁻¹. It is divided into two regions, the functional group region and the fingerprint region. The fingerprint region is different for each molecule just like a fingerprint is different for each person. Two different molecules may have similar
functional group regions because they have similar functional groups, but they will always have a different fingerprint region. The fingerprint region extends from about 1450 cm\(^{-1}\) to 400 cm\(^{-1}\) and it has many absorption bands which makes it quite complex. The IR spectra of all the flavonoids and isoflavonoids show absorption bands in the region 1500-1600 cm\(^{-1}\) due to aromatic rings, along with a carbonyl band at 1620-1670 cm\(^{-1}\).\(^{96}\) The carbonyl absorption does not appear in flavanoids, isoflavonoids pterocarpanoids and chalconoids. The presence of hydroxyl groups in hydroxyflavonoids is evidenced by absorption in the region 3300-3450 cm\(^{-1}\).\(^{97}\) Absorption at 925 cm\(^{-1}\) is indicative of a methylenedioxy group and the presence of gem dimethyl group is indicated by the appearance of a band at 1400 cm\(^{-1}\).\(^{98}\) The glycosidic nature of a flavonoid is reflected by broad bands at 3250 and 1650 cm\(^{-1}\).\(^{99}\) However, although these absorption bands are present in most flavonoid glycosides, they may also occur in the spectra of polyhydroxyflavonoids.

### 1.3.3. Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance (\(^1\)H and \(^{13}\)C NMR) spectroscopy is well-resolved method of analysis for organic compounds, because in many cases it provides a way to determine an entire structure using one set of analytical tests. NMR spectroscopy is used by chemists and biochemists to investigate the properties of organic molecules (natural as well as synthetic), although it is applicable to any kind of sample that contains nuclei possessing spin. The intramolecular magnetic field around an atom in a molecule changes the resonance frequency, thus giving access to details of the electronic structure of a molecule. The use of NMR spectroscopy on the sciences has been substantial because of the range of information and the diversity of samples, including solutions and solids. Different functional groups are obviously distinguishable, and identical functional groups with differing neighboring substituent still give distinguishable signals.

In 1964-65, two groups of workers, Waiss et al.\(^{100}\) and Mabry et al.\(^{101}\) independently investigated the usefulness of trimethylsilyl ether derivatives for obtaining NMR spectra of flavonoids which were insoluble in CDCl\(_3\). The most detailed and systematic studies of the \(^1\)H NMR spectra of flavonoids are documented by Mabry\(^{94,102}\) Batterham and Hightet,\(^{103}\) Okigawa et al.,\(^{104}\) Lewis et al.,\(^{105}\) Massicot and Marathe,\(^{106}\) Miura et al.,\(^{107}\) and Pelter et al.\(^{108}\) These studies have simplified the task of determination of the substitution pattern of flavonoids with the help of NMR.

\(^{23}\)
spectroscopy. $^1$H NMR signals in trimethyl silylated flavonoids$^{178}$ normally occur between 0 and 9 ppm. Most of the trimethylsilyl protons signal of flavonoid TMS ethers occurs between 0.1-0.5 ppm. The chemical shift of the protons of ring A and B prove to be independent of each other but are affected by the nature of ring C. This technique is also helpful to distinguish flavones, isoflavones, flavanones, flavonols and chalcone.$^{94}$$^{13}$C NMR spectral data furnish key information about the carbon backbone of the compound while $^1$H NMR gives information about the structural environment of each proton. It helps in determining the total number of carbons and the number of oxygenated carbons in the skeleton. It also helps in establishing the nature of carbon either it is primary, secondary or tertiary. $^{13}$C NMR occurs over a range of 0-200 ppm downfielded from TMS compared with a range of only 0-10 ppm for $^1$H resonance. In proton decoupled spectra each carbon atom is represented by one line and its chemical shift is determined primarily by the electron density at that carbon atom. The electron resonances at lowest field are generally those of carbonyl carbons and oxygenated aromatic carbons, whereas those at highest field will represent non-oxygenated aliphatic carbons. In case of natural products especially flavonoids it may be of use in special situations. The different types of aglycone are not distinguishable on the basis of aromatic carbon resonances but the chemical shifts of the central three carbon unit are often quite distinctive. The chemical shift values of $^{13}$C NMR for ring C in flavonoids$^{109}$ are given in Table 5.

**Table 5** Chemical shift of $^{13}$C NMR for ring C in flavonoids ($\delta$ in ppm)

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>C-2</th>
<th>C-3</th>
<th>C=O</th>
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<tr>
<td>Flavones</td>
<td>160.5-165</td>
<td>103-111.8</td>
<td>176.3-184</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>149.8-155.4</td>
<td>122.3-125.9</td>
<td>174.5-181</td>
</tr>
<tr>
<td>Flavonols</td>
<td>145-150</td>
<td>136-139</td>
<td>172-177</td>
</tr>
<tr>
<td>Flavanones</td>
<td>75-80.3</td>
<td>42.8-44.6</td>
<td>189.5-196.5</td>
</tr>
<tr>
<td>Chalcones</td>
<td>136.9-145.4$^{*}$</td>
<td>116.6-128.1$^{*}$</td>
<td>188.6-194.6</td>
</tr>
<tr>
<td>Aurones</td>
<td>146.1-147.7</td>
<td>111.6-111.9</td>
<td>182.5-182.7</td>
</tr>
</tbody>
</table>

* For chalcone C-2 and C-3 represents C-β and C-α, respectively.

The chemical shift values $^1$H and $^{13}$C NMR ($\delta$, ppm) of a isoflanonoid (5,6,7-trimethoxy-3-(3',4',5'-trimethoxyphenyl)-4H-chromen-4-one) and chalcone (isoliquiritigenin-4,4'-dimethyl ether) nucleus of our interest in the context of the
compounds isolated in the present work are shown in the following table (Table 6 and 7).

![Chemical structures](image)

**Table 6** $^1$H NMR spectral data of representative isoflavone and chalcone ($\delta$ in ppm)

<table>
<thead>
<tr>
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<th>Assignment of protons</th>
<th>Chalcone</th>
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<tr>
<td>H-2</td>
<td>7.86 (s)</td>
<td>H-2,6</td>
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<td>H-6</td>
<td>6.62 (d, J=2.5 Hz)</td>
<td>H-3,5</td>
<td>6.95 (d, J=9 Hz)</td>
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<tr>
<td>H-8</td>
<td>6.66 (d, J=2.5 Hz)</td>
<td>H-3’</td>
<td>6.45 (d, J=2.5 Hz)</td>
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<tr>
<td>H-5’</td>
<td>6.79 (s)</td>
<td>H-5’</td>
<td>6.51 (dd, J=2.5,9Hz)</td>
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<tr>
<td>3×OCH$_3$</td>
<td>3.84, 3.85, 4.01 (s)</td>
<td>H-6’</td>
<td>7.81 (d, J=9Hz)</td>
</tr>
<tr>
<td>5-OH</td>
<td>13.5 (s)</td>
<td>H-α</td>
<td>7.49 (d, J=16Hz)</td>
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<td>7-OH</td>
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<td>H-β</td>
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<td>4’-OH</td>
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<td></td>
<td></td>
<td>2’-OH</td>
<td>13.56 (s)</td>
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**Table 7** $^{13}$C NMR spectral data of representative isoflavone and Chalcone ($\delta$ in ppm)

<table>
<thead>
<tr>
<th>Assignments of Carbons</th>
<th>Isoflavone</th>
<th>Assignments of Carbons</th>
<th>Chalcone</th>
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<tr>
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<tr>
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<td>4</td>
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<td>7</td>
<td>162.1</td>
<td>6</td>
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<td>8</td>
<td>94.0</td>
<td>α</td>
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<td>156.6</td>
<td>β</td>
<td>143.9</td>
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<tr>
<td>2’</td>
<td>146.0</td>
<td>2’</td>
<td>166.3</td>
</tr>
<tr>
<td>3’</td>
<td>148.8</td>
<td>3’</td>
<td>101.0</td>
</tr>
</tbody>
</table>
1.3.4. Mass Spectrometry

Mass spectrometry (MS) is an analytical technique that ionizes chemical species and sorts the ions based on their mass to charge ratio. It proves to be essential tool for the identification and quantification of natural products and synthesized compounds, primarily because of its speed, sensitivity, selectivity and its versatility in analyzing solids, liquids and gases.\(^{110,111}\) It has become an indispensable apparatus to the modern organic chemists by providing investigators with molecular formulae, isotopic profiles and fragmentation data that are especially useful for structure elucidation. Mass spectroscopy has been successfully employed for the structure determination of flavonoids. The mass spectrum consists of a series of signals each of which represents a charged fragment of the parent flavonoid produced by electron impact within the spectrophotometer. The molecular ion normally appears as a major peak in the MS of aglycones and must be an even mass number due to the presence of only oxygen, carbon, and hydrogen atoms. Characteristics fragments in the MS originate by fission of the M\(^+\) ion into A and B ring derived fragments. These fragmentations usually involve one of the two competing pathways, I is Retro-Diels Alder and II one is normal. The dominant pathway is determined by the aglycone type, although on occasion neither pathway produces significant fragments. The common fragmentation process of isoflavone is shown in Scheme 1 using 5,6,7-trimethoxy-3-(3',4',5'-trimethoxyphenyl)-4H-chromen-4-one (96)\(^{112}\) as a typical example. It showed M\(^+\) at m/z 360. The fragment ions at m/z 345, 330 and 315 were due to the successive loss of three methyl groups. The fragment ions at m/z 332 and 329 are due to the loss of CO and OCH\(_3\), respectively. The Retro-Diels-Alder (RDA) cleavage representing ring-A at m/z 153, 152 and ring-B at m/z 208, 207 and 178 suggested the presence of two hydroxyl group in ring-A and one hydroxyl and three methoxy group in ring-B. Further the common fragmentation process of chalcone have shown in Scheme 2 using isoliquiritigenin-4,4'-dimethyl ether (97)\(^{94}\) as a typical example.
**Scheme 1** Mass fragmentation pattern of representative isoflavone

**Scheme 2** Mass fragmentation pattern of representative chalcone
It showed M$^{+}$ at m/z 284. The fragment ions at m/z 269 and 254 are corresponding to the loss of methyl group from molecular ion peak and from the fragment m/z 269. The fragment ion at m/z 256 showed the loss of carbonyl group from molecular ion peak. Other fragmentation peaks have shown in scheme.

1.4. Biological Studies

1.4.1. DNA binding studies

Binding studies of chemical entities with DNA are important in the development of molecular probes and new therapeutic reagents. These interactions play a central role in rational drug designing and simultaneously DNA sequence recognition by these drugs has been of great interest. Studies have demonstrated that DNA is the primary target of anticancer drugs. Molecules can bind to DNA through a series of interactions like coordination by the DNA bases, intercalation and non-covalent interaction including hydrogen bonding between the coordinated ligands and phosphate oxygen atoms of the sugar-phosphate group.

1.4.2. Structural features of duplex DNA

Double-helical DNA consists of two complementary, anti-parallel, sugar-phosphate poly-deoxyribonucleotide strands which are associated with specific hydrogen-bonding between nucleotide bases. The backbone of these paired strands called the helical grooves, within which the edges of the heterocyclic bases are exposed. B-form of DNA duplex is biologically important and it is characterized by a shallow-wide major groove and a deep-narrow minor groove.

1.4.3. Interaction of DNA-duplex with small organic molecules

1.4.3a. Covalent interaction of duplex-DNA with small organic molecules

Cancer chemotherapy was found as agents that interact with DNA or alkylate it, and such compounds continue to be clinically important today. Alkylating agents are involved in reaction with the preferential N-7 position of guanine and N-3 of adenine in DNA this leads to interference in DNA replication. In the first mechanism, an alkylating agent attaches alkyl groups to DNA bases. This alteration results in the DNA being fragmented by repair enzymes in their attempts to replace the alkylated bases. A second mechanism by which alkylating agents cause DNA damage is the formation of cross-bridges, bonds between two stands of DNA. In this process, two
bases are linked together by an alkylating agent that has two DNA-binding sites. Cross-linking prevents DNA from being separated for synthesis or transcription. The third mechanism of action of alkylating agents causes mis-pairing of the nucleotides leading to mutations. Another well known covalent DNA binder used as an anticancer drug is cis-platin (cis-diammine-dichloroplatinum), which makes an intra/interstrand cross-link with the nitrogens on the DNA bases and used extensively in testicular, ovarian, head, and neck cancers. The early success of cis-platin as an anticancer drug has led to the development of other less toxic derivatives such as carboplatin. However, these agents are mostly non-specific in their action.

1.4.3b. Non-covalent interactions of small molecules with duplex-DNA

(i) Duplex-DNA intercalators:
Molecules that bind to double-stranded DNA by intercalative mode have been significantly used as drugs. The binding of these molecules to DNA is characterized by insertion of planar aromatic rings between the DNA base pairs. This interaction can be quite strong and the stability of intercalation complexes is governed by van der Waals, hydrophobic and electrostatic forces. The two major types of intercalation-binding modes are: (1) classical intercalation and (2) threading intercalation. Binding by the classical mode is studied by DNA staining dye ethidium bromide and antimalarial drug quinacrine. An important contributor to the binding affinity of ethidium bromide and quinacrine to DNA is the stacking interaction of the respective heteroaromatic rings with the DNA base pairs. Intercalation preferentially occurs at G/C-rich sequences (CpG sites), because these sequences get unstacked easily. Intercalators generally cause more significant distortion of the native conformations of DNA, a factor that contributes to the disruption of protein binding. As threading intercalators typically have two side chains on opposite sides of a planar aromatic ring system, the process of complex formation with DNA is more complicated. In such cases, one of the side-chains must slide through the intercalation cavity in order to form the complex. Favourable interactions of the side-chains with both the major and minor grooves contribute to the complex stability of the threading intercalators.

(ii) Duplex-DNA groove-binding molecules:
Groove binders are another major class of small molecules that bind to DNA and play an important role in drug development. In this case molecules can bind to both the major or minor groove of DNA. Due to the dimensional difference, the major grooves
are the site for binding of many DNA interacting proteins. Proteins can recognize and bind to DNA by reading the sequence information in either groove, but most often by major groove recognition. However, nonpeptidyl compounds show a reverse preference, they bind with the minor groove, thus potentially allowing simultaneous major-groove recognition by proteins. Duplexes that are made up of polypurine-polypyrimidine sequences can be read by oligomers that bind in the major groove and form hydrogen bond with bases of the purine strand. The amenability of the minor-groove to bind with small molecules has led many investigators to focus on this aspect. It has been speculated that the evolution of antibiotic minor-groove binders that target the DNA of competing organisms is related to the more attractive dimensions of the minor groove for small molecules. Minor-groove binding usually involves greater binding affinity and higher sequence specificity than that of intercalator binding. The forces that dominate small molecule-minor-groove binding interactions are electrostatic, van der Waals, hydrophobic and hydrogenbonding. A number of crystal structure analysis and NMR studies of Hoechst 33258 complex to various oligonucleotide duplexes containing stretches of AT base pair have been reported. Design of low molecular mass compounds, which bind with high affinity and specificity to pre-determined DNA sequences that are 10-16 base pairs long, is a key issue in chemical biology. Hence the first strategy is to generate new DNA targetable compounds, e.g. the groove-binding agents.

1.4.2. Molecular docking studies

Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The motive of ligand-protein docking is to predict the predominant binding mode (s) of a ligand with a protein of known three-dimensional structure. Docking methods typically use an energy-based scoring function to identify the energetically most favorable ligand conformation when bound to the target. Successful docking methods explore high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings. Docking can be used to perform virtual screening on large libraries of compounds, rank the results, and propose structural hypothesis of how the ligands inhibit the target, which is invaluable in lead optimization. The general hypothesis is that lower energy scores represent better protein-ligand bindings compared to higher energy values. Therefore, molecular docking can be formulated as an optimization problem, where the task is to
find the ligand-binding mode with the lowest energy. In our experiment, rigid molecular docking studies were performed to predict the binding modes of compounds with DNA. The target protein/DNA used in the study has been downloaded from protein data bank (PDB ID: 1BNA). Mol files were converted into PDB format using Avogadro 1.0.1. Energy minimization and molecular optimization of structures were done using Arguslab 4.0.1. Geometry optimization was carried using AM1 (Austin Model 1), semiempirical quantum mechanics force field in Arguslab 4.0.1. and Discovery studio 4.0 software have also been used to predict the possible binding orientations of compounds with target DNA or protein.

1.4.3. Antioxidant studies

Antioxidants play an important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced antioxidants like vitamin C, vitamin E, carotenes, phenolic acids etc. have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Free radicals are an atom or molecule that bears an unpaired electron and is extremely reactive, capable of engaging in rapid change reaction that destabilize other molecules and generate many more free radicals. In plants and animals these free radicals are deactivated by antioxidants. These antioxidants act as an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. The harmful free radicals such as hydroxyl, peroxyl and the superoxide anion are constantly being produced as a result of metabolic reactions in living systems. Several diseases caused by free radicals have been reported such as atherosclerosis, cancer, liver cirrhosis, diabetes, etc. and the scavenging effects of these free radicals by the chemical compounds have great potential in ameliorating these disease progresses.

A simple, rapid and inexpensive method to measure antioxidant capacity of any compounds involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity. The DPPH assay method is based on the reduction of DPPH, a stable free radical.
radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol.\textsuperscript{138} This free radical is stable at room temperature and it reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry,\textsuperscript{138} so it can be useful to assess various products at a time. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH, it reduced to the DPPH-H and as consequence the absorbance decreased from the DPPH.\textsuperscript{139} Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured.\textsuperscript{140} More the decolorization, more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug.\textsuperscript{141} When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present).\textsuperscript{142}

1.4.4. Cytotoxicity

Plants are used as food sources, some of them may have mutagenic or genotoxic potential.\textsuperscript{143} Numerous research studies have recently focused on both pharmacology and toxicity of medicinal plants used by humans.\textsuperscript{144} The toxicity of the plants may originate from different contaminants or from plant chemical compounds that are part of the plant. Around half of the anticancer drugs currently used in clinical trials are of natural origin, and it has been estimated that about 60\% of new chemical entities (NCEs) introduced in the recent years were natural products or were derived from a natural lead compound. Therefore, the screening of traditional medicinal plants has great importance to identify new medicinal plants and to isolate new cytotoxic compounds for life threatening diseases like cancer. Various assays are used for the detection of toxicity of herbal extracts based on different biological models, such as \textit{in vivo} assays on laboratory animals. However, recent studies employed efforts for alternative biological assays that include species of \textit{Artemia} (\textit{A. salina}, \textit{A. franciscana} and \textit{A. urmiana}. These toxicity tests are considered a useful tool for preliminary assessment of toxicity.\textsuperscript{145} Brine shrimp (\textit{A. salina}) is most extensively studied of the \textit{Artemia} species, estimated to represent over 90\% of the studies in which \textit{Artemia} is
used as an experimental test organism. The Brine Shrimp Toxicity Assay was proposed and developed by Michael et al.\textsuperscript{146} and later adapted by Vanhaecke et al.,\textsuperscript{147} Meyer et al.,\textsuperscript{148} and Sleet and Brendel.\textsuperscript{149} Brine Shrimp Lethality Assay (BSLA) has been applied as an alternative bioassay technique to screen the toxicity of plant extracts\textsuperscript{148,150} toxicity of heavy metals,\textsuperscript{151} toxicity of cyanobacteria\textsuperscript{152} and algae,\textsuperscript{153} toxicity of nanoparticles,\textsuperscript{154} as well as screening of marine natural products.\textsuperscript{155} It is capable of detecting various bioactivities present in crude extracts of medicinal plants and has been used as an indicator for general toxicity and as a guide for the detection of antitumor and pesticidal compounds.\textsuperscript{156}
1.5. REFERENCES

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