SYNOPSIS
SYNOPSIS

The Thesis entitled “Investigation on Bioactive Compounds through The Stereoselective Total Synthesis of Stagonolide D, Development of New Synthetic Methodologies and Chemical Examination of Jatropha multifida” consists of five chapters.

CHAPTER-I: Introduction to bioactive natural compounds

The term macrocycle refers to medium- and large-ring compounds, with respectively, 8-11 and 12 or more atoms in the ring. Macrocyclic structures that have one or more ester linkages are generally referred to as macrolides or macrocyclic ring lactones. In some cases, macrocyclic lactams have also been described as macrolides. Originally macrolides denoted a class of antibiotics derived from species of Streptomyces and containing a highly substituted macrocyclic lactone ring aglycone with few double bonds and one or more sugars, which may be amino sugars, non-nitrogen sugars or both. To our knowledge the largest naturally occurring macrolides are the 60-membered quinolidomycins and the largest constructed macrolide is the 44-membered swinholide. Isolation and biological activities of some of these natural products are discussed in this chapter.

CHAPTER-II: Stereoselective total synthesis of stagonolide D

Nonenolides (ten membered lactonic compounds) are common naturally occurring secondary metabolites. Stagonolides are recent examples of these compounds. Various stagonolides have
been isolated from liquid and solid cultures of *Stagonospora cirsii*, a fungal pathogen obtained from *Cirsium arvense*. Some of these natural products have been examined to exhibit antibacterial, antifungal and phytotoxic properties.

Microbial phytotoxins are used as tools for envisaging new molecular sites of action not discovered by the traditional approaches of herbicide discovery. Moreover, there is little overlap between the sites of action of phytotoxins and those of traditional or commercial herbicides. The newer sites would be useful in overcoming the current herbicide resistance problems encountered in weeds. These attributes of microbial phytotoxins have been found to satisfy the complex set of questions put forth by crop protection research groups; and this has influenced research by institutions and industry. With advances in chemical technology and biotechnology, this strategy is becoming less time consuming. Traditionally most investigators were concerned with the isolation, characterization and mode of action of phytotoxins from plant pathogens of crop plants.

*Stagonospora cirsii*, a fungal pathogen isolated from *Colpoda arvense* and proposed for its biocontrol, produces phytotoxic metabolites in liquid and solid cultures. Recently, stagonolide D 1, a compound of this group with interesting phytotoxic property was isolated in 2008 by Antonio and co-workers from a solid culture of *Stagonospora cirsii* and characterized as a new nonenolide by using spectroscopic methods.
In continuation of our efforts towards the synthesis of biologically active naturally products, we initiated a program towards the stereoselective total synthesis of stagonolide D 1. The retrosynthetic analysis of stagonolide D 1 revealed that its both the core fragments, the olefinic alcohol 2 and the olefinic acid 3 can be generated from commercially available D-mannitol 4 (Scheme 1).

Scheme 1. Retrosynthetic analysis of stagonolide D (1)

Synthesis of fragment 2:
The present synthesis of the fragment 2 was initiated by converting D-mannitol 4 into the chiron aldehyde 6 which was subjected to 2C-Wittig olefination with triethyl phosphonoacetate to furnish an intermediate α,β-unsaturated ester 7. The so formed ester was converted into its corresponding alcohol 8 using DIBAL-H which was later protected as benzyl ether 9. Deprotection of acetonide of compound 9 using AcOH:H₂O (6:4) furnished viscous diol 10 which was reacted with PivCl in the presence of Et₃N and DMAP in CH₂Cl₂ when its primary hydroxyl group was protected to furnish the product 11 in high yield.

Treatment of 11 with MsCl using Et₃N and DMAP followed by reaction with methanolic K₂CO₃ afforded the α-epoxide 13. Reduction of this compound 13 with DIBAL-H produced the compound 14 having α-OH group. The free hydroxyl group of 14 was protected as TBDPS ether to form 15 which on subsequent benzyl deprotection using lithium naphthalenide generated the epoxy alcohol 16. The compound 16 was utilized for sharpless asymmetric epoxidation using Ti(Oipr)₄, (+)-DIPT and TBHP in CH₂Cl₂ at -20 °C to yield the epoxy alcohol 17 which was purified for further steps. Compound 17 underwent oxidation with Dess Martin periodinane (DMP) to the corresponding aldehyde (unstable) which on 1C-Wittig olefination with triphenyl phosphonium methyl iodide yielded the olefinic epoxide 18. The TBDPS ether group of 18 was deprotected with TBAF in THF to furnish the desired olefinic alcohol 2 (Scheme 2).
Synthesis of fragment 3:
The second fragment 3 was also prepared from D-mannitol 4 which was converted it into α,β-unsaturated ester 7. The ester so formed was subjected to hydrogenation to form compound 19. Deprotection of acetonide of compound 19 using AcOH:H₂O (6:4) furnished compound 20. Both the hydroxyl groups of this diol 20 were protected by reacting it with TESCl in the presence of DMAP in CH₂Cl₂ to afford the compound 21. The primary TES ether group of 21 was then selectively oxidised under Swern conditions and the resulting aldehyde 22 underwent 1C-Wittig olefination by treatment with triphenyl phosphonium methyl iodide to give the olefinic compound 23 (Scheme 3).
Scheme 3

The ester group of 23 was hydrolysed with LiOH. 8H$_2$O in THF-MeOH-H$_2$O (3:1:1) to yield the olefinic acid 3 (not isolated) which was subsequently coupled with olefinic alcohol 2 in the presence of DCC and DMAP in CH$_2$Cl$_2$.

The resulting diene 24 was then subjected to ring closing metathesis (RCM) using Grubbs' second generation catalyst 25 to produce the nonenolide 26 which was purified by column chromatography. Finally the deprotection of the TES ether group of 26 with HF-pyridine in THF afforded stagonolide D 1 (Scheme 4). The physical and spectral properties of this compound was found to be identical to those reported for the natural product (Scheme 4). The structure of the products were determined from their spectral [IR, $^1$H NMR, $^{13}$C NMR and ESIMS] data and these data for 1 were identical to those reported for the natural product.
In conclusion, a simple route to the second stereoselective total synthesis of stagonolide D is achieved by using Sharpless asymmetric epoxidation (SAE), Ring-closing metathesis (RCM) reactions as the key steps.

CHAPTER-III: Development of new synthetic methodologies using heterogeneous catalysts

This chapter has been further divided into two sections
Section A: Development of new synthetic methodology using silica supported sodium hydrogen sulfate

Development of new synthetic methodologies which involve construction of synthons for the preparation of bioactive molecules is an important subject of synthetic organic chemistry. Nowadays people are developing the methods by using inexpensive materials to yield the desired products selectively in large amounts. The catalyst, NaHSO$_4$.SiO$_2$ is an inexpensive reagent, easy to prepare, highly reactive, possess greater selectivity and works under mild conditions.

3.1. Rapid, efficient and selective conjugate addition of thiols to $\alpha$, $\beta$-unsaturated carbonyl compounds using silica supported sodium hydrogen sulfate (NaHSO$_4$.SiO$_2$) under solvent-free conditions

The conjugate addition of thiols to $\alpha$, $\beta$- unsaturated carbonyl compounds is an important reaction in biosynthetic processes and also in organic synthesis of bioactive compounds such as the calcium antagonist diltiazem. A few methods have been reported for synthesis of these compounds are associated with certain drawbacks such as the harsh reaction conditions, longer reaction times, unsatisfactory yields (especially with aliphatic aldehydes), uses of expensive reagents, toxic solvents.

As a part of our research on the development of novel useful synthetic methodologies we have developed the silica supported sodium hydrogen sulfate (NaHSO$_4$.SiO$_2$) catalyzed efficiently for 1, 4-addition of thiols to $\alpha$, $\beta$- unsaturated carbonyl compounds to afford
the corresponding thia-Michael addition products in excellent yields under solvent-free conditions at room temperature (Scheme 5).

A series of β- sulfido carbonyl derivatives were prepared from various α, β- unsaturated carbonyl compounds and thiols following the procedure mentioned in the scheme. The conversion underwent at room temperature and the products were formed within a short period of time (2-5 min). No additional solvent was required. Both open-chain and cyclic α, β- unsaturated carbonyl compounds afforded the desired products smoothly. The products were prepared from α, β- unsaturated ketones and also from α, β- unsaturated esters.

The structures of the products were determined from their spectral [IR, 1H NMR and ESIMS] data.

In conclusion, we have developed a convenient and efficient method for the preparation of β- sulfido carbonyl compounds by NaHSO$_4$.SiO$_2$ catalyzed conjugate addition of thiols to α, β- unsaturated carbonyl compounds at room temperature. The operational simplicity, mild heterogeneous reaction conditions, rapid conversion, application of an inexpensive catalyst, solvent-free reaction and excellent yields and selectivity are the notable advantages of the present method.
Section B: Development of new synthetic methodology using Amberlyst-15

3.2. Stereoselective synthesis of β-amino ketones: A three component Mannich-type reaction of aromatic aldehydes, anilines and cyclohexanone using Amberlyst-15

Amberlyst-15 is an important heterogeneous catalyst used in various chemical transformations. β-amino carbonyl compounds are important synthons for construction of various biologically active compounds. They also exist as structural units in various bioactive natural products.

Here we developed a convenient and efficient method for the stereoselective synthesis of β-amino ketones using Amberlyst-15 as a catalyst. We have observed that the Mannich-type reaction of aromatic aldehydes 30, anilines 31 and cyclohexanone 32 can efficiently were carried out using this catalyst at room temperature to form the corresponding β-amino ketones 33a and 33b (Scheme 6).

![Scheme 6](image)

86-92 %

13 Examples
A series of β-amino ketones were prepared from various aromatic aldehydes and anilines. The reaction was carried out at room temperature, conversion was completed within 1–2.5 h and the products were formed in high yields. The diastereoselectivity of the reaction was high and the formation of the anti-isomers was favoured. The ratio of the anti- and syn-isomers were determined from the $^1$H NMR spectra of the products. The values of the coupling constant ($J$) between the vicinal protons $\alpha$ and $\beta$ to the carbonyl group for anti-isomer (ca 7.5 Hz) is higher than those for syn-isomer (ca 4.5 Hz).

The above reaction of β-amino ketones proceeds through the following reaction mechanism (Scheme 7).

Scheme 7

The products were characterized from their [IR, $^1$H NMR and ESIMS] spectral data.

In conclusion, we have developed a convenient and efficient protocol for one-pot synthesis of β-amino ketones by three-component Mannich-type reaction of aromatic aldehydes, anilines and cyclohexanone in the presence of Amberlyst-15 and the method is associated with several advantages such as simple experimental
procedure (column chromatography), utilization of a heterogeneous catalyst, milder conditions, short reaction times, high yields, good diastereoselectivity and reusability of the catalyst.

CHAPTER-IV: Development of new synthetic methodologies using homogeneous catalysts

**This chapter has been further divided into two sections**

Section A: Development of new synthetic methodology using para-Toluene Sulfonic Acid

4.1. An efficient thiocyanation of Indoles using para-Toluene Sulfonic Acid

*p*-Toluene Sulfonic Acid (*p*-TSA) is commercially available and is a very cheap chemical with stability, a safe and inexpensive reagent. *p*-TSA has recently been utilized in various organic transformations. Thus, on the basis of economic and ecological considerations the reagent *p*-Toluene Sulfonic Acid is highly valuable and used as a catalyst.

Thiocyanation of aromatic and heteroaromatic compounds is a useful reaction in organic synthesis as this reaction initiates the direct introduction of sulphur into the molecules. Aryl and heteroaryl thiocyanates are important intermediates for the synthesis of sulphur containing heterocycles. Thiocyanate group can also be converted into other sulphur-bearing groups. They are particularly useful for producing drugs and pharmaceuticals.

Here we have observed that the thiocyanation of indoles can efficiently be carried out by treatment with ammonium thiocyanate in
the presence of \textit{para}-Tolune Sulfonic Acid at room temperature (\textbf{Scheme 8}).

\begin{align*}
\text{34} & \quad \xrightarrow{\text{NH}_2\text{SCN, p-TSA, MeOH, r.t., 30-45 min}} \quad \text{35} \\
& \quad 85-91\% 
\end{align*}

\textbf{Scheme 8} \quad 9 \text{ examples}

A series of indoles containing electron-donating as well as electron-withdrawing group underwent the conversion smoothly. The derivatives of indole having substituents in the aromatic ring as well as 2-methyl indole and \textit{N}-methyl indole were equally applied. In each case only the mono-thiocyanation product was formed. The reaction was completed within 30-45 min. Indoles The reaction is highly regioselective forming 3–thiocyanano derivaties as the products.

The structures of the products were determined from their spectral [IR, \textsuperscript{1}H NMR and ESIMS] data.

In conclusion, we have developed a simple, convenient and efficient method for high-yielding regioselective thiocyanation of indoles by treatment with ammonium thiocyanate using \textit{para}-toluene sulfonic acid at room temperature. The simple experimental procedure, high yields and short reaction times are the notable advantages of the protocol.

\textbf{Section A: Development of new synthetic methodologies by one-pot multicomponent approach}
Multi-component reactions (MCRs) are a promising and vital field of chemistry because the synthesis of complicated molecules can be achieved in a very fast, efficient, and time saving manner without the isolation of any intermediate. As a result, it requires a minimum effort, which minimizes the environmental loading.

Section B: Development of new synthetic methodology using 2,4,6-trichloro 1,3,5-triazine (TCT)

4.2. Metal-free multicomponent synthesis of α-amino phosphonates using 2,4,6-trichloro 1,3,5-triazine

α-Amino phosphonic acid and their derivatives possess various valuable medicinal properties including anticancer, anti-HIV and antibacterial activities. They are also applied as enzyme inhibitors and peptide mimics.

TCT has recently been utilized in various organic transformations and is a safe and inexpensive reagent. It has been applied here, for the first time, for the preparation of α-amino phosphonates.

Here we described a facile synthesis of α-amino phosphonates 38 by the multicomponent reaction of aldehydes 36 and amines 37 with triethyl phosphate in the presence of a catalytic amount of 2,4,6-trichloro-1,3,5-triazine (TCT, cyanuric chloride) at room temperature (Scheme 9).
A series of α-amino phosphonates were efficiently prepared from different aldehydes and amines. Both the aromatic and aliphatic aldehydes were applied to prepare these compounds. The aromatic aldehydes and the anilines used in this conversion contained electron-donating as well as electron-withdrawing groups. Various functional groups such as halogen, hydroxyl, nitro and ester remained unchanged. The conversion was completed within 0.5-1 h and α-amino phosphonates were formed in excellent yields.

TCT is known to react with the water generated by the reaction of aldehydes and amines (during the formation of imines) or with incipient moisture to release hydrochloric acid. This acid subsequently activates the >C=N− bond of the imines to produce α-amino phosphonates (Scheme 10).

The structures of the products were characterized from their [IR, \(^1\)H NMR, \(^13\)C NMR and ESIMS] spectral data.
In conclusion, we have developed a simple and efficient method for the synthesis of α-amino phosphonates by a one-pot reaction of aldehydes, amines and triethyl phosphite in the presence of 2,4,6-trichloro-1,3,5-triazine (TCT) at room temperature. The mild reaction conditions, simple experimental procedure, utilization of an inexpensive and readily available catalyst, excellent yields, rapid conversion and application of a metal-free safe reagent are the notable advantages of the present method.

CHAPTER-V: Chemical Examination of Jatropha multifida

Plants and plants derived natural products have been in use to cure the human sufferings from time immemorial. The searching for pharmacologically active agents obtained by screening natural sources such as plant extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human ailments. Today, plants continue to retain historical significance as important sources of novel compounds useful directly as medicinal agents or as lead compounds for synthetic or semisynthetic structure modification and optimization as biochemical or pharmacological probes.

The genus Jatropha is very important in the family of Euphorbiaceae as the plants belong to the genus are reputed in the Indian Ayurvedic System of medicine for their medicinal properties and also contain various metabolites, which are chemically and biologically interesting. Due to containing of latex this family is also
known as spurge family, which is having 300 genera and 800 species. The plants of the genus are familiar for important medicinal values and for producing various compounds, which possess interesting pharmacological actions. Earlier investigation on the latex of *Jatropha multifida* afforded some diterpenoids, triterpenoids, coumarins, coumarino-lignoids, flavonoids, alkaloids, cyclic peptides, phenolics, glucsides and steroids. The peptides were shown to inhibit selectively the classical pathway of human complement activation. However the chemical examination of the other parts of the plant has not yet been reported. So we undertook a detailed investigation on the stems of the plant. The previously reported medicinal activities and isolated constituents of *Jatropha* genus have been reviewed to initiate the chemical investigation on *Jatropha multifida*.

Various species of *Jatropha* are known for their medicinal properties. Almost all the parts of *Jatropha gossypifolia* species have been used in the Indian Ayurvedic system. The latex of the plant employed to cure ulcers and leprosy. The ether extract of the roots showed antibiotic activity. The decoction of the leaves has been used for stomachache, general diseases and a blood purifier. The seeds of *Jatropha curcas* possess poisonous and purgative properties and are used as purgative. In Brazil the seeds are considered as antihelmintic. Jatrogressidion, the main diterpene of *Jatropha grossidentata* was tested against *Leishmania* and *Trypanosoma cruzi* strains in vitro as well as against *Leishmania amazonensis* in vivo. It showed a strong in
vitro leishmanicidal and trypanocidal activity with IC$_{50}$ 0.75 and 1.5-5.0 µg/mL.

We have presently described the extraction details, isolation and structure elucidation of the compounds from *Jatropha multifida*. Here, the stem of the plant material has been investigated and chemical examination on its extract afforded one new lathyrane diterpenoid along with seven known components include jatrophone, 15-epi-(4E)-jatrogrossidentadion, 4E-jatrogrossidentadion, 6-O-acetyl-(4E)-jatrogrossidentadion, 15-O-acetyl japodagrone, Cleomiscosin A, Pectolinargenin. The compounds were isolated from the CHCl$_3$: MeOH (1:1) extract of the plant material. The structures of the compounds were established from their elemental analysis and detailed spectroscopic ($^1$H NMR, 2D NMR, IR and MS) data. The known compounds were identified from their physical and spectral data and by comparing the values with those in the literature and/or by direct comparison with authentic samples available in the laboratory. Some of these compounds are known to possess interesting biological properties.

**Table 1:** Biological activity of compounds isolated from the stems of *Jatropha multifida*

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>Structure</th>
<th>Remark</th>
<th>Reported/identified activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Jatrophone</td>
<td>39</td>
<td>Known compound</td>
<td>Antitumor/ Cytotoxic</td>
</tr>
<tr>
<td>B</td>
<td>15-epi-4(E)-</td>
<td>40</td>
<td>Known compound</td>
<td>Cytotoxic</td>
</tr>
</tbody>
</table>
Thus the present investigation has lead to the isolation of several bioactive naturally occurring compounds from *Jatropha multifida*.
The structure of new molecule 15-\textit{epi-(4E)}-jatrogressidentadione acetate 46 has been confirmed by detailed analysis of its spectral (IR, $^1$H-NMR, $^{13}$C-NMR, COSY, HMBC, HSQC, NOESY and MS) data. It has been found to be a new lathyrane diterpenoid.