Chapter-I: Introduction to cancer chemotherapeutics
This chapter deals with introduction to cancer, natural products in folk medicine, natural product derived anticancer agents in clinical use, natural product derived anticancer agents in clinical development, drug delivery vehicles in cancer chemotherapy.

Chapter-II: Isolation, design and synthesis of anticancer compounds based on natural product scaffolds.
This chapter is sub-divided into three sections.

Section A: Isolation of podophyllotoxin and design, synthesis and biological evaluation of 4β-[1,2,3-triazol-1-yl] podophyllotoxin derivatives as anticancer compounds.
Podophyllotoxin is a most abundant naturally occurring cyclolignan, mainly isolated from Podophyllum peltatum and podophyllum hexandrum. Podophyllotoxin has cathartic, antirheumatic and antiviral properties but its anticancer activity has proved to be the most attractive for researchers. Podophyllotoxin is known as an antimicrotubule agent acting at the colchicine-binding site on tubulin. Due to severe toxicity of podophyllotoxin, it is not being used as anticancer drug, but its semi synthetic derivatives etoposide and teniposide (Figure-1) are clinically useful drugs against various cancers, including small cell lung cancer, testicular carcinoma, lymphoma, and Kaposi’s sarcoma. The chemical modifications that led to etoposide, teniposide and other derivatives, also lead to the change in the mechanism of action of these ligands wherein podophyllotoxin act as antimicrotubule agent whereas its aforementioned derivatives act as topoisomerase-II inhibitors. These derivatives block the catalytic activity of DNA topoisomerase-II by stabilizing a cleavage enzyme-DNA complex in which the DNA is cleaved and covalently linked to the enzyme. However, the therapeutic use of etoposide & teniposide is often hindered by problems such as acquired drug-resistance and poor water solubility. To get more potent analogues and to overcome drug resistance recently several complex and more diverse analogues like Etopophos, GL-331, TOP-53, NK611, NPF etc. have been synthesised (Figure-1). Etopophos, is a water-soluble prodrug of etoposide, is readily converted in vivo to the active drug etoposide and exhibits similar pharmacological and pharmacokinetic profiles that of etoposide. NK-611, NPF and GL331 are presently under clinical trial. According to structure activity relationship (SAR) of podophyllotoxin, trans-lactone, 4β-substituted and 4’-demethyl moieties were essential to maintain the anticancer activity as topoisomerase-II inhibitors. Particularly 4β-N-substituted
derivatives of podophyllotoxin gained much importance owing to their improved cytotoxicity.

Keeping in view of the biological importance of 1,2,3-triazoles as anticancer compounds and SAR of podophyllotoxin, we initiated a programme on the design and synthesis of triazole derivatives of podophyllotoxin. Our molecular modelling studies revealed that 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxins are good topoisomerase-II inhibitors. Using Cu catalysed 1,3-dipolar cycloaddition (click chemistry protocol) of 4β-azido podophyllotoxins with various terminal alkynes, various 4β-[(4-alkyl)-1,2,3-triazol-1-yl] podophyllotoxin and 4β-[(4-aryl)-1,2,3-triazol-1-yl] podophyllotoxin derivatives have been synthesised and presented here. All the compounds synthesised by click chemistry protocol were screened for their in vitro anticancer activity against a panel of six human cancer cell lines. Most of the 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxins exhibited higher cytotoxicity than etoposide. Based on these encouraging results, a focused library of 4β-[(4-alkyl)-1,2,3-triazol-1-yl] picropodophyllins has been synthesized using click chemistry approach and 4β-[(5-alkyl)-1,2,3-triazol-1-yl] podophyllotoxins employing Cp*RuCl(PPh3)2 catalysed azide and alkyne 1,3-dipolar cycloaddition strategy. All these podophyllotoxin derivatives were screened for in vitro cytotoxicity against a panel of human cancer cell lines.
Figure-1: Structures of podophyllotoxin & its derivatives.
Scheme-1: Synthesis of 4β-[(4-alkyl)-1,2,3-triazol-1-yl] podophyllotoxins using click chemistry approach.

Scheme-2: Synthesis of 4β-[(5-alkyl)-1,2,3-triazol-1-yl] podophyllotoxins using ruthenium catalyst.

Scheme-3: Synthesis of various phenyl acetylenes using Corey-Fuch reaction.
Scheme-4: Synthesis of 4β-[(4-aryl)-1,2,3-triazol-1-yl] podophyllotoxins using click chemistry approach.

Scheme-5: Synthesis of 4β-[(4-alkyl)-1,2,3-triazol-1-yl] picropodophyllins using click chemistry approach.
Section B: Isolation of parthenin and design, synthesis and biological evaluation of spiro-isoxazolidine derivatives of parthenin as anticancer compounds.

Parthenin is a major sesquiterpene lactone psuedoguianolide isolated from *Parthenium-hysterophorus* L (*Compositae*). It contains α-methylene-γ-butyrolactone and pentenone moieties along with other functionalities and five chiral centers. Parthenin exhibit several biological activities like anticancer, antibacterial, antiamoebic and antimalerial properties. However, the compound is toxic. Thus, despite numerous biological activities of parthenin, no concrete SAR model for this molecule has been established till date. Even though several groups in India and abroad have been working on the structural modification of parthenin, none of these reports reveal a focused and rational approach to the modification of parthenin in order to develop a SHAL with better anticancer activity. The cytotoxicity, as many other biological activities of parthenin type of molecules, is known to be mediated by the presence of potentially alkylant structure elements capable of reacting covalently with biological nucleophiles in Michael addition type of fashion, thereby inhibiting a variety of cellular functions which directs the cells into apoptosis. In parthenin there are two alkylating structures, one is the exocyclic double bond and the other is the endocyclic one. To establish the role of exo/endocyclic double bonds towards the anticancer activity, a strategy to selectively react one of these double bonds has been devised. Out of few chemical transformational possibilities available to achieve the above goal, the present approach involves the selective addition of dipole, i.e., nitrone to exocyclic double bond, which should also enhance the activity due to the introduction of basic nitrogen atom into the structural framework. Thus, keeping in view the Lipinski’s rule together with the possibility to introduce nitrogen bearing (alkaloid type) structural moiety, better secondary leads could be possibly derived applying this strategy. Both endo- as well as the exocyclic double bond are active double bonds, but because of steric hindrance offered by the substitution at the cyclopentenone ring, nitrone cycloadds selectively to the exocyclic double bond alone. In this section, nitrone cycloaddition to the exocyclic double bond of parthenin to generate a focused library of novel spiro isoxazolidines is presented. By screening the anticancer activity of these novel spiro derivatives we can easily establish the pharmacological importance of cyclopentenone ring or the α-methylene-γ-butyrolactone ring; thereby we can establish the SAR of the molecule unequivocally. For this I had isolated the parthenin from *Parthenium-
*hysterophorus* *L* according to literature procedures and synthesized a series of isaxazolidine derivatives. The entire spiro isoxazolidine derivatives were screened for their possible anticancer activity against a panel of human cancer cell lines.

![Figure-2: parthenin](image)

**Figure-2: parthenin**

**Scheme-6: synthesis of spiro-isoxazolidine derivatives of parthenin**

**Section-C: Design, synthesis and biological evaluation of 1,2,3-triazole derivatives of santonin as anticancer compounds.**

Santonin has anthelminthic, anti-inflammatory activity and antimicrobial activity. Santonin is a drug which was widely used in the past as an anthelminthic. Due to severe side effects and the development of many safer deworming drugs, santonin has largely fallen out of use. Recently several santonin analogues reported to have anticancer activity. Sesuitepene lactones generally contain α-methylene-γ-lactone or α, β-unsaturated pentenone moieties as structural feature, according to the structure activity relationship of sesquiterpenoids, anticancer and other biological activities are due to the alkylation of biological nucleophiles on these double bonds. In view of the importance of this scaffold in developing new
anticancer leads through the introduction of double bond at lactone ring and appropriate substitution on the skeleton, a programme has been initiated to generate 1,2,3-triazole derivatives of santonin employing click chemistry approach. Santonin was first reduced using \( \text{H}_2/\text{PtO}_2 \) to get \( 3\beta\)-hydroxy-4:5:7-\( \alpha \)(H),6:11\( \beta \)(H)-eudesman-6:12-olide (75 %) and \( 3\alpha \)-hydroxy-4:5:7-\( \alpha \)(H),6:11\( \beta \)(H)-eudesman-6:12-olide (20 %). This hydroxy compound (\( 3\beta \)-hydroxy-4:5:7-\( \alpha \)(H),6:11\( \beta \)(H)-eudesman-6:12-olide) was tosylated then reacted with \( \text{NaN}_3 \) to get the azido compound. This azido derivative was treated with \( \text{PhSeBr} \) & \( \text{H}_2\text{O}_2 \) according to literature procedure to create a double bond adjacent to lactone ring. All the 1,2,3-triazole derivatives of santonin were screened for their anticancer activity \textit{in vitro} against a panel of human cancer cell lines.

\[
\text{santonin} \xrightarrow{\text{H}_2/\text{PtO}_2} \begin{array}{c}
\text{HO}^\prime \\
\text{HO}^\prime \\
\end{array} + \begin{array}{c}
\text{HO} \\
\text{HO} \\
\end{array}
\]

1. \( \text{TsCl/Py} \)
2. \( \text{NaN}_3 \)

\[
\begin{array}{c}
\text{N}_3^\prime \\
\end{array} \xrightarrow{\text{PhSeBr}} \begin{array}{c}
\text{N}_3 \\
\end{array}
\]

1. \( \text{PhSeBr} \)
2. \( \text{H}_2\text{O}_2 \)

\[
\text{CuSO}_4.5\text{H}_2\text{O} \\
\text{Sodium ascorbate} \\
t\text{-BuOH}:\text{H}_2\text{O} (1:2)
\]

Scheme-7: Synthesis of 1,2,3-triazol derivatives of santonin
Chapter-III: Design and synthesis of tumor homing peptide conjugates and isolation of bioactive peptides from Indian traditional food.

This chapter is sub-divided into two sections.

Section A: Design and synthesis of tumor homing peptide conjugates.

Peptides that are home to tumors are called as tumor homing peptides. Tumor cells express many molecules on their surface that distinguishes them from normal cells. Tumor blood vessels are distinct from normal vessels. In addition to being tortuous, uneven in diameter, and leaky, tumor vessels express various cell surface and extracellular matrix proteins that normal vessels do not. The expression of many of these peptides/proteins in tumor vessels is associated with angiogenesis, and they are often functionally important in that process. Tumors also contain lymphatic vessels, and many tumors produce growth factors that stimulate lymphangiogenesis. Lymphatics are not necessary for tumor growth but are important conduits of metastasis. Like tumor blood vessels, tumor lymphatics can also express specific molecular markers. Tumor homing peptides selectively recognizes these markers and goes to tumor site. We can use these tumor homing peptides as carriers for tumor specific drug delivery. Anchoring anticancer drug to tumor homing peptide with suitable linker will improve the selectivity, efficiency and decrease the dosage of the drug.

Using phage display method several tumor homing peptides have been identified and several successful anticancer drug-tumor peptide conjugates have been reported.

Podophyllotoxin shows strong cytotoxic activity against various cancer cell lines, but due to its severe toxicity to normal cells it has not being used as anticancer drug. Conjugation of podophyllotoxin and its derivatives with tumor homing peptides will increase the specicity and reduce the toxicity to normal cells. In view of this I had selected NVVRQ, CDTRL, CGKRK and CREKA homing peptides from literature. NVVRQ specifically bound to PC-3M-1E8, breast cancer MDA-MB-435S, lung cancer PG-BE1, and gastric cancer MKN-45 cells. CGKRK preferentially homed to Skin carcinoma and dysplastic skin (K14-HPV16), MDA-MB-435, MMTV-PyMT breast carcinomas, C8161 melanomas. CDTRL preferentially homed to Skin carcinoma and dysplastic skin (K14-HPV16), MMTV-PyMT breast carcinomas, C8161 melanomas. CREKA specially bound to PyMT tumors and MDA-MB-435. Here I had synthesized various podophyllotoxin & epipodophyllotoxin tumor homing peptide conjugates using Fmoc chemistry. Tumor homing peptides NVVRQ, CGKRK,
CDTRL and CREKA are synthesized on solid-phase (2-chloro trityl chloride) and conjugated to podophyllotoxin & epipodophyllotoxin with succinyl linker. Podophyllotoxin on reaction with DMAP in DCM gives succinylated podophyllotoxin quantitatively.

Epipodophyllotoxin on reaction with DMAP in DCM gives succinylated epipodophyllotoxin quantitatively.

NVVRQ-podophyllotoxin was synthesized manually on 2-chloro trityl chloride resin in a Merrifield reactor. Fmoc-Gln(Trt)-OH dissolved small amount of dry DMF and DIPEA (4 eq related Fmoc-amino acid) were introduced into the Merrifield reactor and shaken for 3h under N₂ atmosphere. Then the resin was endcapped with DCM/MeOH/DIPEA (7:2:1, 2 x 20 mL) and washed with CH₂Cl₂/MeOH (5 x 20 mL) alternatively, DMF (20 mL), CH₂Cl₂/MeOH (3 x 20 ml) and finally with dry Et₂O. Then the resin was dried in vacuum. A small portion of the resin (2 mg) was suspended in 3 mL 20 % piperidine solution, in DMF and shaken for 10 minutes. The solution was decanted and loading of Fmoc-Gln(Trt)-OH on resin was determined by it’s UV analysis using the following conversion factor.

\[
\text{Loading} = \frac{0.181818 \times \text{absorbance of peak at 290nm} \times 10}{\text{Weight of resin in mg} \times \text{volume of piperidine/DMF solution in mL}}
\]

Complete Fmoc cleavage of the resin-bound amino acid was done by shaking it in 20% piperidine in DMF solution for 30 minutes. Then the resin was washed with CH₂Cl₂/MeOH alternatively (5 times), DMF, CH₂Cl₂/MeOH and finally with dry Et₂O. Then Fmoc-Arg(pmc)-OH (5 eq to the loading of previous amino acid) was coupled to resin bound amino acid using HBTU/HOBt/DIPEA in DMF. The washing cycle was repeated. Loading was checked as described above and Fmoc was deprotected for next coupling. Using the same strategy amino acids Fmoc-Val-OH, Fmoc-Val-OH, Fmoc-Asn-OH and succinylated
podophyllotoxin were coupled. Finally protecting groups and solid support were removed using TFA/DCM/thioanisole to obtain pure NVVRQ-podophyllotoxin conjugate.

Table-1: Few tumor homing peptides

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Name</th>
<th>Target tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDCRGDCFC</td>
<td>RGD-4C</td>
<td>several tumor types (including carcinoma, sarcoma, and melanoma)</td>
</tr>
<tr>
<td>CRGDC</td>
<td></td>
<td>Several tumor types</td>
</tr>
<tr>
<td>CRGDKGPDC</td>
<td>iRGD</td>
<td>Several tumor types</td>
</tr>
<tr>
<td>CNGRCVSGCAGRC</td>
<td>NGR</td>
<td>Several tumor types</td>
</tr>
<tr>
<td>CNGRC</td>
<td></td>
<td>Several tumor types</td>
</tr>
<tr>
<td>TAASGVRSMH</td>
<td></td>
<td>B16 melanoma</td>
</tr>
<tr>
<td>LTLRWVGMS</td>
<td></td>
<td>B16 melanoma</td>
</tr>
<tr>
<td>KDEPQRRSARLSAKP-APPKPEPKKKAPKK</td>
<td>F3</td>
<td>MDA-MB-435 (melanoma), HL-60</td>
</tr>
<tr>
<td>CGNKTRRGCC</td>
<td>Lyp-1</td>
<td>MDA-MB-435 (melanoma), MMTV-PyMT (breast), KRI B (osteosarcoma), K14-HPV16 (skin), TRAMP (prostate)</td>
</tr>
<tr>
<td>CNRRRTKAGCC</td>
<td>Lyp-2</td>
<td>K14-HPV16 (skin) tumor + premalignant lesion, K14-HPV16/E2 (cervix) tumor + premalignant lesion</td>
</tr>
<tr>
<td>CNRRRTKAGCC</td>
<td></td>
<td>Premalignant and malignant lesions of the cervix and dysplasias and squamous cell carcinomas of the skin</td>
</tr>
<tr>
<td>CLSDGKRKC</td>
<td>LSD</td>
<td>C8161 (human melanoma), KRI B (osteosarcoma)</td>
</tr>
<tr>
<td>CREAGRKAC</td>
<td>REA</td>
<td>Prostate cancers, MMTV-PyMT (breast), K14-HPV16/E2 (cervical cancer), KRI B</td>
</tr>
<tr>
<td>CAGRRSAYC</td>
<td>AGR</td>
<td>TRAMP (prostate) premalignant lesion</td>
</tr>
<tr>
<td>CRSRKRG</td>
<td>RSR</td>
<td>Angiogenic islets (RIP1-Tag2)</td>
</tr>
<tr>
<td>CKAAKNK</td>
<td>KAA</td>
<td>Pancreatic tumors (RIP1-Tag2)</td>
</tr>
<tr>
<td>CRGRRST</td>
<td>RGR</td>
<td>Pancreatic tumors and angiogenic islets (RIP1-Tag2) Dysplastic skin (K14-HPV16)</td>
</tr>
<tr>
<td>CREKA</td>
<td></td>
<td>PyMT tumors and MDA-MB-435</td>
</tr>
<tr>
<td>CSRPRRSEC</td>
<td></td>
<td>Dysplastic skin (K14-HPV16)</td>
</tr>
<tr>
<td>NVVRQ</td>
<td>TMTP1</td>
<td>PC-3M-1E8, breast cancer MDA-MB-435S, lung cancer PG-BE1, and gastric cancer MKN-45sci</td>
</tr>
<tr>
<td>CGKRK</td>
<td>KRK</td>
<td>Skin carcinoma and dysplastic skin (K14-HPV16), MMTV-PyMT breast carcinomas, MDA-MB-435, C8161 melanomas</td>
</tr>
<tr>
<td>CDTRL</td>
<td></td>
<td>Skin carcinoma and dysplastic skin(K14-HPV16), MMTV-PyMT, C8161 melanomas</td>
</tr>
<tr>
<td>CGTKRKCC</td>
<td></td>
<td>Skin carcinoma (K14-HPV16)</td>
</tr>
</tbody>
</table>
Scheme-8: Synthesis of podophyllotoxin-NVVRQ and epipodophyllotoxin-NVVRQ conjugates

\[ \text{Cl} \rightarrow \text{a} \rightarrow \text{Ph} \rightarrow \text{b, c} \rightarrow \text{d, e} \]

\[ \text{Cl} = 2\text{-chloro tritylchloride} \]
Reagents: a) Fmoc-Gln(Trt)-OH, DIPEA, DMF b) DMF/piperidine c) Fmoc-Arg(pmc)-OH, HBTU, HOBT, DIPEA, DMF d) DMF/piperidine e) Fmoc-Val-OH, HBTU, HOBT, DIPEA, DMF f) DMF/piperidine g) Fmoc-Asn(Trt)-OH, HBTU, HOBT, DIPEA, DMF h) DMF/piperidine i) Succinylated podophyllotoxin, HBTU, HOBT, DIPEA, DMF j) TFA/DCM/thioanisole k) Succinylated epipodophyllotoxin, HBTU, HOBT, DIPEA, DMF l) TFA/phenol/water/thioanisole/1-dodecanethiol (82.5:5:5:5:2.5).

\[ \text{FmocHN} \cdot \text{Cl} \xrightarrow{a} \text{FmocHN} \cdot \text{NH} \cdot \text{O} \cdot \text{Cl} = 2\text{-chloro tritylchloride} \xrightarrow{b, c} \text{FmocHN} \cdot \text{NH} \cdot \text{CO} \cdot \text{NH} \cdot \text{O} \cdot \text{Cl} \xrightarrow{d, e} \text{FmocHN} \cdot \text{NH} \cdot \text{CO} \cdot \text{NH} \cdot \text{O} \cdot \text{Cl} \xrightarrow{f, g, h} \text{FmocHN} \cdot \text{NH} \cdot \text{CO} \cdot \text{NH} \cdot \text{O} \cdot \text{Cl} \]
Reagents: a) Fmoc-Lys(Boc)-OH, DIPEA, DMF  
b) DMF/piperidine  
c) Fmoc-Arg(pmc)-OH, HBTU, HOBT, DIPEA, DMF  
d) DMF/piperidine  
e) Fmoc-Gly-OH, HBTU, HOBT, DIPEA, DMF  
f) DMF/piperidine  
g) Fmoc-Cys(tBu)-OH, HBTU, HOBT, DIPEA, DMF  
h) DMF/piperidine  
i) Succinylated podophyllotoxin,
HBTU, HOBT, DIPEA, DMF  

j) TFA/DCM/thioanisole  
k) Succinylated epipodophyllotoxin, HBTU, HOBT, DIPEA, DMF  
l) TFA/phenol /water/thioanisole/1-dodecanethiol (82.5:5:5:5:2.5).

**Scheme-10:** Synthesis of podophyllotoxin-CDTRL & epipodophyllotoxin-CDTRL conjugates
Reagents:  
a) Fmoc-Leu-OH, DIPEA, DMF  
b) DMF/piperidine  
c) Fmoc-Arg(pmc)-OH, HBTU, HOBT, DIPEA, DMF  
d) DMF/piperidine  
e) Fmoc-Thr(tBu)-OH, HBTU, HOBT, DIPEA, DMF  
f) DMF/piperidine  
g) Fmoc-Asp(tBu)-OH, HBTU, HOBT, DIPEA, DMF  
h) DMF/piperidine  
i) Fmoc-Cys(tBu)-OH, HBTU, HOBT, DIPEA, DMF  
j) DMF/piperidine  
k) Succinylated podophyllotoxin, HBTU, HOBT, DIPEA, DMF  
l) TFA/DMC/thioglycerol.  
m) DMF/piperidine  
n) Succinylated podophyllotoxin, HBTU, HOBT, DIPEA, DMF  
o) TFA/phenol//water/thioglycerol/1-dodecanethiol (82.5:5:5:5:2.5).
Scheme-11: Synthesis of podophyllotoxin-CREKA & epipodophyllotoxin-CREKA conjugates
Reagents: a) Fmoc-Ala-OH, DIPEA, DMF  b) DMF/piperidine  c) Fmoc-Lys(Boc)-OH, HBTU, HOBT, DIPEA, DMF  d) DMF/piperidine  e) Fmoc-Glu(tBu)-OH, HBTU, HOBT, DIPEA, DMF  f) DMF/piperidine  g) Fmoc-Arg(pmc)-OH, HBTU, HOBT, DIPEA, DMF  h) DMF/piperidine  i) DMF/piperidine  j) Fmoc-Arg(pmc)-OH, HBTU, HOBT, DIPEA, DMF  k) Succinylated podophyllotoxin, HBTU, HOBT, DIPEA, DMF  l) TFA/DCM/thioanisole  m) Succinylated epipodophyllotoxin, HBTU, HOBT, DIPEA, DMF  n) TFA/phenol/water/thioanisole/1-dodecanethiol (82.5:5:5:5:2.5).
Section B: Identification of novel anticancer and immunomodulatory peptides from Indian fermented food derived from *Oryza sativa*

Fermentation is one of the oldest and most economical methods of producing and preserving food. In addition, fermentation provides a natural way to enhance essential amino acids, proteins, vitamins, to destroy antinutrients, to enhance aroma, flavours and appearance of the food, to reduce the energy required for cooking and to make a safer product. Fermented foods are good source of bioactive peptides and essential amino acids. Fermentation is consider to be one of the best way to produce bioactive peptides and Several fermented foods based on milk, soybean, fish etc has been studied extensively and identified several peptides having antimicrobial, anticancer, antihypertensive, antioxidant, immunomodulatory, and opioid activities. Moreover, some peptides are multifunctional and can exert more than one of the effects mentioned above.

India has several traditional fermented food and beverages like idli, dosa, lassi, dahi, naan, dhokla, uthappam, jann, daru etc. Among these idli and dosa are very much popular and has great hystory. The microbiological, physical and biochemical changes of idli and dosa during fermentation and its nutritive values are almost same. The lactic acid bacteria *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Lactobacillus delbrueckii*, *Lactobacillus fermenti*, *Lactobacillus lactis* and *Pediococcus cerevisiae* have been found to be responsible for the fermentation process, although *L. mesenteroides* and *S. faecalis* are considered to be the microorganism essential for leavening of the batter and for acid production in idli and dosa. The yeasts *Geotrichum candidum*, *Torulopsis holmii*, *Torulopsis candida* and *Trichosporon pullulans* have also been identified in idli and dosa fermentation.

Till date no reports are available revealing the presence of bioactive peptides available in rice based fermented food idli and dosa. The identification of novel bioactive compounds will contribute towards better understanding the benefits of these foods to enhance health and quality of life. In view of the importance of these bioactive peptides, Maldi mass spectral analysis, Ms/MS and *De Novo* sequencing of peptides followed by Data base search (MASCOT) has been undertaken for the peptide enriched fraction of fermented food batter used for the preparation of Idli and dosa and the presence of novel peptides were confirmed, arising out of enzymatic cleavage of proteins present in the feed stock. Four peptides RLEKNSTTSDDSSPLRA (1849 Da), VDDVIPESFTAGSEYKSG (1901 Da),
SRLEKNSSTTSVPSLRA (1936 Da) and TPRRLSPLPSVAPLSAEPLL (2114 Da) were identified originating from *Oryza sativa*. The peptide enriched fraction exhibited moderate anticancer activity at higher concentrations and potential immunomodulatory activity.