COMPARATIVE PHYLOGENETIC APPROACH FOR DISCOVERING ALTERNATIVE SOURCES OF CAMPTOTHECIN – AN ANTICANCER DRUG

NUPUR SONI1 AND ALOK MITTAL2

1Department of Bioinformatics, MANIT, Bhopal, India, 2Department of Chemistry, MANIT, Bhopal, India.

Received: 6 July 2011, Revised and Accepted: 2 Nov 2011

ABSTRACT

Camptothecin is an essential precursor of semisynthetic chemotherapeutic agents for cancers throughout the world. In spite of the rapid growth of market demand, camptothecin raw material is still harvested by extraction from Camptothecin acuminata and Nothapodytes foetida. But still its total synthesis is not cost-effective. However, these natural resources are being threatened day by day due to the destructive collection of wight bark and Chinese happy tree seeds for camptothecin production. Thus, for the stable supply of camptothecin and for the development of more potent anticancer compounds, alternative sources for the biosynthesis of camptothecin are urgently anticipated. The computational approaches for prediction of the alternatives are fast and economical and therefore can be used to complement the existing wet lab techniques. In this paper an attempt has been made to find alternative sources for camptothecin by using Comparative Phylogenetics approach based on molecular, structural and physicochemical level so as to lessen the threat on the existing species. The analysis was performed using enzymes physicochemical data, domain organization and sequence data. Dendogram was generated through physicochemical data whereas phylogenetic trees (based on sequence alignment and structural alignment) were generated through sequence data. Identification of domain families of homologous protein was done to analyze their structural homology relationship. Consequently after comparison, three different organisms, Arabidopsis thaliana, Ricinus communis (castor oil plant) and Oryza sativa (Rice) were observed to be related to Camptothecin plant (Chinese Happy Tree) sequentially, physicochemically and structurally, thus providing alternatives for Camptothecin production.

Keywords: Camptothecin, Comparative phylogenetics, Structural alignment, Physiochemical properties, Dendogram, Phylogenetic tree

INTRODUCTION

Plants have been used for treating various diseases of human beings and animals since time immemorial. They maintain the health and vitality of individuals, and also cure diseases, including cancer without causing toxicity. More than 50% of all modern drugs in clinical use are natural products, many of which have the ability to control cancer cells. A recent survey shows that more than 60% of cancer patients use vitamins or herbs as therapy. Therefore, natural protection against cancer has recently been receiving a great deal of attention not only from cancer patients but, surprisingly, from physicians as well. Camptothecin, a pentacyclic quinoline alkaloid, has been discovered as a potential anticancer agent through an extensive screening by the U.S. National Cancer Institute. It is a potent broad spectrum antitumor agent which is of interest due to a variety of camptothecin derivatives currently being used in a variety of clinical protocols. Camptothecin functions by effectively converting DNA topoisomerase I into a DNA damaging poison, causing it to irreversibly bind DNA midway through its catalytic action, leading to a double-stranded break when encountered by a replication fork. Camptothecin and its derivatives are active against carcinomas of the stomach, colon, head, neck and bladder.

At present, semisynthetic water-soluble camptothecin analogs, topotecan and irinotecan, are prescribed as clinical antitumor drugs throughout the world. CPT derivatives that exhibit remarkable action, leading to a double-stranded break when encountered by a replication fork, and their structural homology relationship, consequently after comparison, three different organisms, Arabidopsis thaliana, Ricinus communis (castor oil plant) and Oryza sativa (Rice) were observed to be related to Camptothecin plant (Chinese Happy Tree) sequentially, physicochemically and structurally, thus providing alternatives for Camptothecin production.

MATERIALS AND METHODS

Enzymes regulating camptothecin biosynthesis pathway and their BLAST search.

Except for a few undefined steps, the complete camptothecin biosynthetic pathway was elucidated and many genes encoding certain enzymes, which regulate its biosynthesis pathway, have been cloned and characterized in Ophiopogon pumila. Though...
camptothecin is structurally classified as a quinoline alkaloid, it belongs to a family of monoterpenoid indole alkaloids from the viewpoint of biosynthesis.²

Table 1: List of 5 reported enzymes of camptothecin biosynthetic pathways

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Accession no.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>strictosidine synthase (STR)</td>
<td>OpSTR; EC 4.3.3.2</td>
<td>²⁵</td>
</tr>
<tr>
<td>tryptophan decarboxylase (TDC)</td>
<td>OpTDC; EC</td>
<td>²⁶</td>
</tr>
<tr>
<td>NADPH:cytochrome P450 reductase (CPR)</td>
<td>OpCPR; EC</td>
<td>²⁶</td>
</tr>
<tr>
<td>selcolganin synthase (SLS)</td>
<td>SLS; EC 1.3.3.9</td>
<td>²⁷,²⁸</td>
</tr>
<tr>
<td>geraniol 10-hydroxylase (GI10H)</td>
<td>G10H; EC</td>
<td>²⁶</td>
</tr>
</tbody>
</table>

Nucleotide and protein sequences of each of the 5 enzymes were retrieved from the Genbank accession Numbers, which were then subject to a BLAST search against the non-redundant database with the e-value inclusion threshold, set to 0.005. By using an option available in the BLAST program to select a particular organism, the search was restricted to plants. In this way potential homolog of enzymes were selected.

Data preparation and characterization

Sequence data, physicochemical data and structural data of enzymes was collected, processed and analyzed for phylogenetic analysis.

A) Physiochemical data

The protein sequence of each enzyme was subjected to ExPASy tool, ProtParam to obtained its physiochemical property such as No. of AAs, No. of Atoms, Molecular weight, Isoelectric point, Positive charge residues(Asp + Glu), Negative charge residues(Arg + Lys), Alphabetic index, Instability index, Hydrophathy index and Extinction coefficient.

B) Sequence based data

In order to prepare data for phylogenetic tree analysis, entire protein sequence of each set of homologous enzymes was subjected to site based alignment. MEGAl ⁴.0 software was used to analyse the Constant sites(C), Variable sites (V) and Singleton sites(S). Then overall similarity of the alignments was calculated by the Feature similarity score (FSS).

Feature similarity score (FSS) = Constant site + Singleton site / Total sites

C) Structure based Data

To study the phylogenetic relationship, the protein sequences of each set of homologus enzymes was subjected to structure based alignment through Expresso (3D-Coffee) - Regular (http://tcoffee.vital-it.ch/) ²³ which aligns the templates using structural aligners like sap or TMalign. Thereafter, these structure based alignments are used as a template for realigning the original sequences. Moreover, family identification of sequence homologues was done through FISH Server (Family identification with Structure Anchored HMM) (http://babel.ucpb.univ.s.fr/fish/) ²⁴

Phylogenetics Tree and Dendogram generation

A) Dendogram generation

The physicochemical properties of each enzyme and their homologous is collected and calculated by ExPASy tool, ProtParam. Thereafter, distance measurement and linkage method are chosen for clustering of the physicochemical data. Finally, the statistical software Minitab is use to compute and generate dendogram.

B) Tree generation on basis of sequence alignment and structural alignment

The sequence alignment obtained from the MEGA software is subjected to phylogenetic tree generation. There are different methods of tree generation. Which method should be adopted is a great question. Thus, depending upon the nature of data (built alignment), the following categories were made:

- If data has high similarity (high FSS) i.e. greater than 75% - character based method (MP).
- If data has moderate similarity (medium FSS) i.e. less than 75% and more than 50% - distance based method (NJ, UPGMA)
- If data has lower similarity (low FSS) i.e. less than 50% - ML method

After observation of data, Cut off (%) is planned.

The structural alignments obtained from the EXPRESSO (3D Coffee) are then used for phylogenetic tree generation using PHY.FL (cgi-www.cs.au.dk/cgi-chill/phyfl/gf) ²⁵ This program produces colored phylogenetic tree from the parenthesis Newick format phylogeny obtained from EXPRESSO (3D Coffee) RESULTS

Similarity search of enzymes involved in the biosynthesis pathway helps in the prediction of other biological sources that contains the same enzymes and thus provides an useful approach for discovering alternative and cost effective source for the camptothecin biosynthesis.

Output from physicochemical data

Physicochemical data of strictosidine synthase (STR) and its homologous which is calculated by ExPASy tool, ProtParam is shown in Table-2. STR is one of the 5 known enzymes involved in camptothecin biosynthetic pathway.

Table 2: Physicochemical data of STR enzyme and its homologs

<table>
<thead>
<tr>
<th>Name of protein enzyme</th>
<th>N</th>
<th>M</th>
<th>MW</th>
<th>PI</th>
<th>NG</th>
<th>PS</th>
<th>I1</th>
<th>EC</th>
<th>Al</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB060531H.1-stricosidine synthase [Ophiopogon pumila]</td>
<td>358</td>
<td>5418</td>
<td>3889.06</td>
<td>5.15</td>
<td>45</td>
<td>30</td>
<td>34.11</td>
<td>44600</td>
<td>83.02</td>
<td>-0.19</td>
</tr>
<tr>
<td>ACF21007.1-stritosidine synthase [Ophiopogon japonica]</td>
<td>353</td>
<td>5432</td>
<td>3928.53</td>
<td>5.75</td>
<td>40</td>
<td>33</td>
<td>31.52</td>
<td>4584</td>
<td>82.55</td>
<td>-0.2</td>
</tr>
<tr>
<td>ARZ79473.1-stritosidine synthase [Mitragyna speciosa]</td>
<td>352</td>
<td>5407</td>
<td>3875.15</td>
<td>5.65</td>
<td>38</td>
<td>31</td>
<td>27.14</td>
<td>47330</td>
<td>81.93</td>
<td>-0.19</td>
</tr>
<tr>
<td>P68175.1-stritosidine synthase [Rauvolfia serpentina]</td>
<td>344</td>
<td>5332</td>
<td>3816.2</td>
<td>5.19</td>
<td>41</td>
<td>28</td>
<td>35.61</td>
<td>47330</td>
<td>84.68</td>
<td>-0.18</td>
</tr>
<tr>
<td>P68174.1-stritosidine synthase [Rauvolfia manillii]</td>
<td>342</td>
<td>5305</td>
<td>3795.97</td>
<td>5.19</td>
<td>41</td>
<td>28</td>
<td>35.4</td>
<td>47330</td>
<td>84.88</td>
<td>-0.19</td>
</tr>
<tr>
<td>P81417.2-stritosidine synthase [Catharanthus roseus]</td>
<td>352</td>
<td>5412</td>
<td>3909.37</td>
<td>5.14</td>
<td>43</td>
<td>29</td>
<td>47.42</td>
<td>48820</td>
<td>73.04</td>
<td>-0.29</td>
</tr>
<tr>
<td>CAN78856.1-hypothetical protein [Vitis vinifera]</td>
<td>600</td>
<td>6410</td>
<td>64700.1</td>
<td>5.23</td>
<td>63</td>
<td>52</td>
<td>30.94</td>
<td>52830</td>
<td>93.27</td>
<td>0.048</td>
</tr>
<tr>
<td>ACU19372.2-unknown [Glycine max]</td>
<td>336</td>
<td>5105</td>
<td>36360.2</td>
<td>8.32</td>
<td>23</td>
<td>29</td>
<td>38.23</td>
<td>52900</td>
<td>867.9</td>
<td>-0.09</td>
</tr>
<tr>
<td>NP_177540.3-stritosidine synthase [Arabidopsis thaliana]</td>
<td>329</td>
<td>4931</td>
<td>34666.3</td>
<td>9.66</td>
<td>23</td>
<td>39</td>
<td>17.95</td>
<td>24410</td>
<td>83.22</td>
<td>0.053</td>
</tr>
<tr>
<td>AAB40593.1-stritosidine synthase [Arabidopsis thaliana]</td>
<td>335</td>
<td>4978</td>
<td>35293.1</td>
<td>5.76</td>
<td>31</td>
<td>29</td>
<td>26.49</td>
<td>29910</td>
<td>86.12</td>
<td>0.07</td>
</tr>
<tr>
<td>XP_00235297.1-stritosidine synthase [Ricinus communis]</td>
<td>375</td>
<td>5946</td>
<td>42292.2</td>
<td>6.26</td>
<td>47</td>
<td>45</td>
<td>34.71</td>
<td>62340</td>
<td>91.28</td>
<td>-0.21</td>
</tr>
<tr>
<td>AAR07254.1-stritosidine synthase [Oryza sativa japonica]</td>
<td>480</td>
<td>7507</td>
<td>53706.2</td>
<td>8.75</td>
<td>59</td>
<td>65</td>
<td>41.85</td>
<td>62340</td>
<td>79.65</td>
<td>-0.29</td>
</tr>
</tbody>
</table>

(Abbreviations- N=No. AAs, M= No. of atoms, MW= Molecular Weight, PI= Isoelectric Point, NG= (Asp+ Glu) PS= (Arg+Lys), I1= Instability Index, EC= Extension Coefficient, Al= Alphabetic Index, H= Hydropathicity)
Similarly, physiochemical data was collected for other 4 enzymes and dendrogram was generated from these data for each enzyme by using statistical software Minitab.

**Output from Sequence based data**

Site based alignment was done to find similarity between all the 5 enzymes involved in the pathway.

From the table 3 it is clear that, out of 5 enzymes of biosynthetic pathway, enzymes with higher FSS are zero, moderate FSS is one and low FSS are four, subsequently distance based and maximum likelihood tree are constructed by MEGA and Phylip software. The phylogenetic analysis reveals that the trees are unrooted.

**Output from Structure based data**

The structural alignment of the protein sequences through EXPRESSO (3D Coffee) (figure1) and Domain superfamily identification from FISH revealed the presence of following homologous domains as in table 4.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of Enzyme</th>
<th>Conserved/ Constant site</th>
<th>Variable sites</th>
<th>Singleton sites</th>
<th>Total sites</th>
<th>FSS index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Strictosidine synthase</td>
<td>54</td>
<td>452</td>
<td>100</td>
<td>668</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>Tryptophan decarboxylase</td>
<td>40</td>
<td>394</td>
<td>82</td>
<td>543</td>
<td>40.8</td>
</tr>
<tr>
<td>3</td>
<td>NADPH-cytochrome P-450 reductase</td>
<td>297</td>
<td>412</td>
<td>155</td>
<td>801</td>
<td>56</td>
</tr>
<tr>
<td>4</td>
<td>Secologanin synthase</td>
<td>3</td>
<td>658</td>
<td>197</td>
<td>970</td>
<td>20.6</td>
</tr>
<tr>
<td>5</td>
<td>Geraniol 10-hydroxylase</td>
<td>82</td>
<td>449</td>
<td>68</td>
<td>553</td>
<td>27.14</td>
</tr>
</tbody>
</table>

**Table 3:** Characterization of all 5 known enzymes of camptothecin biosynthetic pathway through MEGA 4.0

Fig. 2 shows phylogenetic trees of Enzymes- Strictosidine synthase (STR) from physiochemical data, molecular data and structural data.

Two phylogenetic trees and dendogram belonging to each group are shown in (figure-2, 3 and 4).
Table 4: Domain Family Information for homologues of enzyme Strictosidine synthase (STR)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>No. of Domains</th>
<th>Class</th>
<th>Fold</th>
<th>Superfamily</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>STR Ophiorrhiza pumila</td>
<td>1</td>
<td>All beta proteins</td>
<td>6-bladed beta-propeller</td>
<td>Calcium-dependent phosphotriesterase</td>
<td>SGL-like</td>
</tr>
<tr>
<td>STR Ophiorrhiza japonica</td>
<td>1</td>
<td>All beta proteins</td>
<td>6-bladed beta-propeller</td>
<td>Calcium-dependent phosphotriesterase</td>
<td>SGL-like</td>
</tr>
<tr>
<td>STR Mitragyna speciosa</td>
<td>1</td>
<td>All beta proteins</td>
<td>6-bladed beta-propeller</td>
<td>Calcium-dependent phosphotriesterase</td>
<td>SGL-like</td>
</tr>
<tr>
<td>STSY_RAUMA P68174.1</td>
<td>1</td>
<td>All beta proteins</td>
<td>6-bladed beta-propeller</td>
<td>Calcium-dependent phosphotriesterase</td>
<td>SGL-like</td>
</tr>
<tr>
<td>STSY_CATRO P18417.2</td>
<td>1</td>
<td>All beta proteins</td>
<td>6-bladed beta-propeller</td>
<td>Calcium-dependent phosphotriesterase</td>
<td>SGL-like</td>
</tr>
<tr>
<td>STR Vitis vinifera</td>
<td>2</td>
<td>All beta proteins</td>
<td>6-bladed beta-propeller</td>
<td>1.Calcium-dependent phosphotriesterase 2.NHL repeat</td>
<td>1.SGL-like 2. NHL repeat</td>
</tr>
<tr>
<td>STR Glycine max</td>
<td>3</td>
<td>1,2. All beta proteins 3.Alpha and beta proteins (a+b)</td>
<td>1,2. 6-bladed beta-propeller 3.TBP-like</td>
<td>1.Calcium-dependent phosphotriesterase 2.NHL repeat 3.Bet v1-like</td>
<td>1.SGL-like 2. NHL repeat 3.STAR domain</td>
</tr>
<tr>
<td>STR Arabidopsis thaliana</td>
<td>2</td>
<td>All beta proteins</td>
<td>6-bladed beta-propeller</td>
<td>1.Calcium-dependent phosphotriesterase 2.NHL repeat</td>
<td>1.SGL-like 2. NHL repeat</td>
</tr>
<tr>
<td>STR Arabidopsis thaliana</td>
<td>2</td>
<td>All beta proteins</td>
<td>6-bladed beta-propeller</td>
<td>1.Calcium-dependent phosphotriesterase 2.NHL repeat</td>
<td>1.SGL-like 2. NHL repeat</td>
</tr>
<tr>
<td>STR Ricinus communis</td>
<td>2</td>
<td>All beta proteins</td>
<td>6-bladed beta-propeller</td>
<td>1.Calcium-dependent phosphotriesterase 2.NHL repeat</td>
<td>1.SGL-like 2. NHL repeat</td>
</tr>
<tr>
<td>STR Oryza sativa</td>
<td>1</td>
<td>All beta proteins</td>
<td>6-bladed beta-propeller</td>
<td>Calcium-dependent phosphotriesterase</td>
<td>SGL-like</td>
</tr>
</tbody>
</table>

**Fig. 3:** shows phylogenetic trees of Enzymes- NADPH:cytochrome P450 reductase (CPR) from physiochemical data, molecular data and structural data

(1-Ophiorrhiza pumila, 2-Ophiorrhiza japonica, 3-Mitragyna speciosa, 4-Rauvolfia serpentine, 5-Rauvolfia mannii, 6-Catharanthus roseus, 7-Vitis vinifera, 8-Glycine max, 9-Arabidopsis thaliana, 10-Arabidopsis thaliana, 11-Ricinus communis, 12-Oryza sativa)
Fig. 4: shows phylogenetic trees of Enzymes- Tryptophan decarboxylase (TDS) from Physicochemical data, molecular data and structural data
(1-Ophiirhiza pumila, 2-Centaurium erythraea, 3-Capsicum annuum, 4-Petunia x hybrida, 5-Gossypium hirsutum, 6-Ricinus communis, 7-Vicia sativa, 8-Glycine max, 9-Populus trichocarpa, 10-Arabidopsis thaliana, 11-Papaver somniferum, 12-Oryza sativa, 13-Zea mays)

tryptophan decarboxylase

(1-Ophiirhiza pumila, 2-Ophiirhiza prostrata, 3-Capsicum annuum, 4-Camptotheca acuminata, 5-Catharanthus roseus, 6-Papaver somniferum, 7-Argemone mexicana, 8-Populus trichocarpa, 9-Ricinus communis, 10-Arabidopsis thaliana, 11-Oryza sativa)
DISCUSSIONS

Drugs of plant origin, maintain the health and vitality of individual and also cure various diseases including cancer without causing toxicity. Due to the adverse side effect of synthetic anticancer drugs, plant origin drug like Camptothecin is currently prescribed in the clinical field for cancer treatment. Camptothecin is still obtained by the extensive extraction from its natural resources which may possess a threat to existing species.

In the present study, an attempt has been made to determine the alternative sources of anticancer drug- Camptothecin through comparative phylogenetic analysis of the five known enzymes involved in its biosynthetic pathway. Feature similarity score (FSS) was calculated for molecular data, structural alignment was performed for phylogenetic relationship of domain superfamily, whereas distance measurement and linkage method was studied for dendogram generation. It was observed that-

1. *Ricinus communis*, *Oryza sativa* and *Arabidopsis thaliana* contain all the five enzymes (Enzymes-STR, TDC, CPR, SLS, G10H) of Camptothecin biosynthesis.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Species</th>
<th>Comparative Result</th>
<th>Supported by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strictosidine synthase(STR)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Less supported</td>
<td>Sequence and structural phylogenetic tree</td>
</tr>
<tr>
<td></td>
<td><em>Ricinus communis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oryza sativa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophan decarboxylase (TDC)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Less supported</td>
<td>Structural phylogenetic tree and Physiochemical dendogram</td>
</tr>
<tr>
<td></td>
<td><em>Ricinus communis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oryza sativa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NADPH:cytochrome P450 reductase (CPR)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Well supported</td>
<td>Sequence and structural phylogenetic tree and Physiochemical dendogram</td>
</tr>
<tr>
<td></td>
<td><em>Ricinus communis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oryza sativa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secologanin synthase (SLS)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Well supported</td>
<td>Sequence and structural phylogenetic tree and Physiochemical dendogram</td>
</tr>
<tr>
<td></td>
<td><em>Ricinus communis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oryza sativa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geraniol 10-hydroxylase (G10H)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Less supported</td>
<td>Sequence and structural phylogenetic tree</td>
</tr>
<tr>
<td></td>
<td><em>Ricinus communis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oryza sativa</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Three different organisms, *Arabidopsis thaliana*, *Ricinus communis* and *Oryza sativa*, have been observed to be related to Camptothecin producing plants sequentially, structurally and physiochemically.

CONCLUSION

This result data is of great significant value as it provides highly authentic information about the alternative source of a drug, by considering not only sequence similarity but also physiochemical relatedness and structural homology. Our work provides a new direction to the research in the area of bioprospecting for discovering alternative sources for the valuable compounds thereby improving the quality of products and their cost effectiveness.

ACKNOWLEDGEMENT

We are thankful to Department of Bioinformatics, MANIT, and Bhopal, India for their support and cooperation in the production of manuscript.

REFERENCES


2. Vitis vinifera (STR, TDC, SLS, G10H) and *Populus trichocarpa* (TDC, CPR, SLS, G10H) comprises of four enzymes involved in Camptothecin biosynthesis.

3. *Glycine max* possess three enzymes (STR, CPR, G10H). Whereas, *Capsicum annuum* (TDC, CPR) and *Catharanthus roseus* (TDC, G10H) both possess two-to two enzymes each of the Camptothecin biosynthetic pathway.

4. TDC is found in *Capsicum annuum* and *Papaver somniferum* which are distantly related to *Camptotheca acuminata* and *Ophiopogon pumila* plants.

On the basis of sequence based phylogenetics tree, physiochemical dendogram and structure based phylogenetic tree, two groups were identified:

A) Less supporting (Enzymes- STR, TDC, G10H),

b) Well supporting (Enzymes-SLS, CPR) (as shown in table-5)

Table 5: Comparative result of sequence, structural and physiochemical data based on phylogeny as well as quality of camptothecin enzymes with other plants

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Species</th>
<th>Comparative Result</th>
<th>Supported by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strictosidine synthase(STR)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Less supported</td>
<td>Sequence and structural phylogenetic tree</td>
</tr>
<tr>
<td></td>
<td><em>Ricinus communis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oryza sativa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophan decarboxylase (TDC)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Less supported</td>
<td>Structural phylogenetic tree and Physiochemical dendogram</td>
</tr>
<tr>
<td></td>
<td><em>Ricinus communis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oryza sativa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NADPH:cytochrome P450 reductase (CPR)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Well supported</td>
<td>Sequence and structural phylogenetic tree and Physiochemical dendogram</td>
</tr>
<tr>
<td></td>
<td><em>Ricinus communis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oryza sativa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secologanin synthase (SLS)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Well supported</td>
<td>Sequence and structural phylogenetic tree and Physiochemical dendogram</td>
</tr>
<tr>
<td></td>
<td><em>Ricinus communis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oryza sativa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geraniol 10-hydroxylase (G10H)</td>
<td><em>Arabidopsis thaliana</em></td>
<td>Less supported</td>
<td>Sequence and structural phylogenetic tree</td>
</tr>
<tr>
<td></td>
<td><em>Ricinus communis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Oryza sativa</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


4. Yamazaki Mani; Asano, Takashi; Yamazaki, Yasuyo; Sirikantaramas, Supaart; Sudo, Hiroshi; Saito, Kaz. Biosynthetic system of camptothecin: an anticancer plant product. Pure and Applied Chemistry. 2010; report


Pharmacological investigations on *Boswellia serrata*. Pharmacognosy Rev. 2008; 2: 219


