IN-SILICO BIOPROSPECTING OF THE ENZYMES INVOLVED IN THE BIOSYNTHETIC PATHWAY OF THE ALKALOID BERBERINE AND ITS DISTANCE STUDY THROUGH R

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ABSTRACT

Bioprospecting or biodiversity prospecting is a scientific tool, which provides us a new and successful means with the help of which search for an alternative source having similar properties same as the target. This tool can be used in the search of those sources which are not present in abundance or near to be endangered. Berberine an alkaloid present in plant Berberis aristata is one of the enzyme having various medicinal properties and is near to be endangered due to its exploitation. Chemical structure of the alkaloid berberine, retrieved from a well-known database chemspider. Its biosynthetic pathway was retrieved from BRENDA database. The enzymes present in the biosynthetic pathway of the alkaloid were checked for their homology through BLAST software. Thereafter, with the help of R software various dendrograms were generated for distance study. Hence, with the help of in-silico bioprospecting of enzymes present in the biosynthetic pathway of alkaloid berberine, alternative resources were found.

Index Terms— *Alkaloid, Berberine, Berberis Aristata, BRENDA, BLAST, Chemspider*

I. INTRODUCTION

Our planet earth is a reservoir of everything that anyone can seek for, for the existence of life. In itself it contains solutions to our problems. Our biodiversity, flora-fauna etc. all of them are part of our existence and are interrelated to each other somehow. But instead of saving them, we are exploiting them for our needs. On top of all of them, most widely we exploit various plants, trees, fungi for their medicinal uses which lead them to their extinction. So in order to save these Richie's we practice bioprospecting which helps us to find alternative sources to those particular elements or material which are being widely exploited and are important in various ways [1]. Bioprospecting is a procedural tool which searches for useful biological or organic compounds in micro-organisms, plants, fungi etc. prospecting is the first stage to any physical search of biological elements. Bioprospecting helps us to search for compounds or enzymes which can prove to be very useful to the human kinds and others too. Bioprospecting also plays a vital role in the conservation of various biological or environmental compounds. Bioprospecting is nothing new, it has been practiced from early years of civilization. The people of early years have been using medicines being formed from the various medicinal plant or trees or fungi species [1]. As now the world is advancing in almost every field and aspects of science and technology. The method of bioprospecting has also been developed and advanced. After the identification of

various biosynthetic pathways involving various enzymes discovered by scientist, a much faster and cost effective method is started being practiced and is known as insilico-bioprospecting [1]. In insilico-bioprospecting, insilico term means, work done is computer based. The prospecting which was earlier used in physical or field is now being done in computers with the help of various softwares and databases. Also the insilico techniques can prove to be cost and time effective. These provide higher precision and better quality of experimental data [1]. Berberin*e* an alkaloid found in plant *Berberis aristata*. It is traditionally called as *daru haridra* or Indian barberry is a native to the Himalayas. It has various medicinal properties like anti-pyretic, anti-bacterial, anti-microbial, anti-hepatotoxic, anti-hyperglyceamic, anti-cancer, anti-oxidant and anti-lipidemic agent. All the above properties of alkaloid berberine, is the reason why it's being cut or exploited and is now endangered. This is why we are looking for an alternative source which can easily be found in abundance and have similar phytochemistry as the alkaloid found in *Berberis aristata* [1]. Hence, insilico-bioprospecting is way better than traditional bioprospecting in various terms like cost, time, precision or accuracy, and is a very effective tool for finding alternative resource having similar enzymes with various medicinal properties. This technique can hence help us to save the jewels of our planet and will lead to its betterment.

II. MATERIALS AND METHOD

For finding alternative sources to enzymes involved in biosynthesis of Berberine alkaloid, following publicly available softwares was used

2.1 Chem Spider

It is a database owned by the royal society of chemistry. This database provides us with a fast access to numerable chemical structures along with their properties and other information. Chemical structure of the alkaloid Berberine was retrieved from this vary database as it can be freely accessed [2].

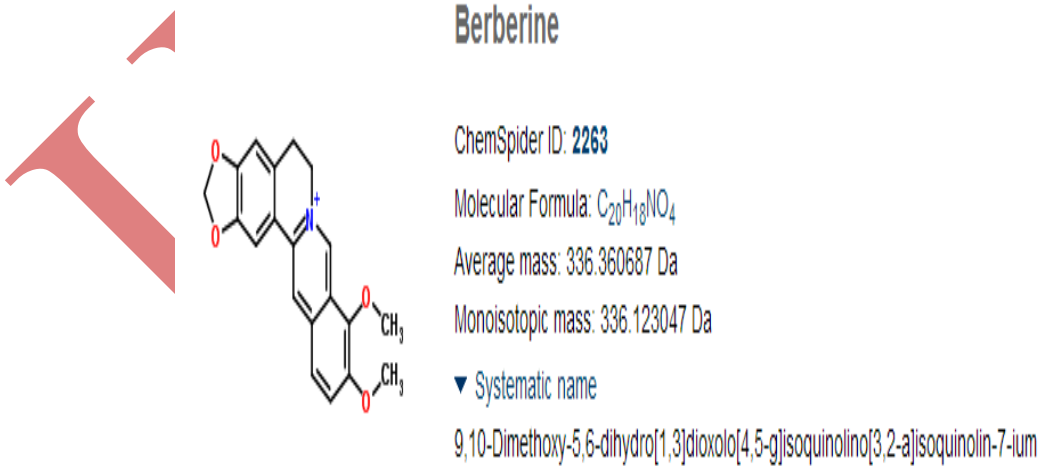

Fig 1. Chemical structure of Berberine

2.2 Brenda

It is a scientific tool used worldwide. This database provides us to access to biosynthetic pathways of various enzymes. Biosynthetic pathway of Berberine was retrieved from this tool. It also gave us the information of the enzymes present in its pathway [3] [4].

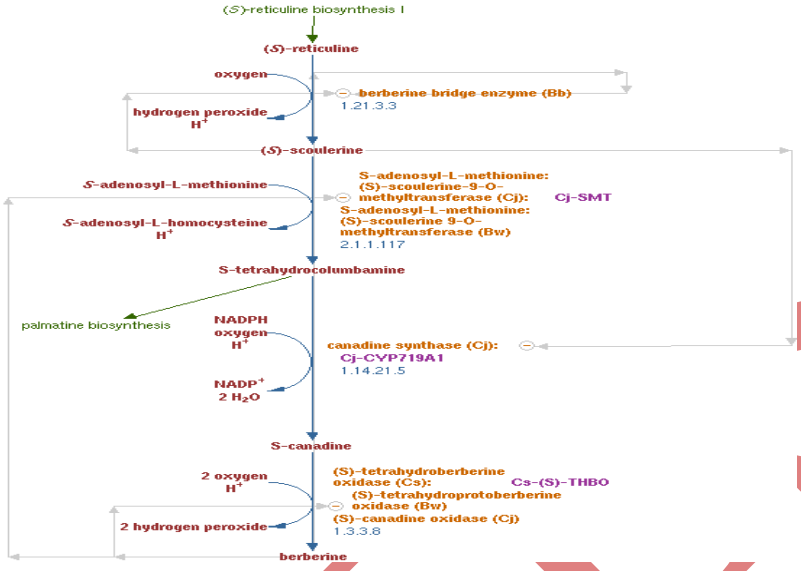

Fig 2. Pathway of Berberine

2.3 Blast

It is a scientific tool used as an algorithm for comparing various biological sequences especially primary sequences such as amino acids, nucleotides etc. BLAST searches for high scoring sequence alignment between the query sequences and the sequences stored in the database using an approach called smith-waterman algorithm. With the help of BLAST tool homologies for the enzyme which were present in the biosynthetic pathway of the alkaloid berberine, were found [4].

2.4 ProtParam

This software provides us with various physical and chemical parameters for a given protein stored in Swiss-prot or TrEMBL or for a user entered sequence in computational form. With the help of this tool the computed parameters of the enzymes like molecular weight, isoelectric point, amino acid component, extinction coefficient, half-life etc. were retrieved which are further used for the distance study [6][7].

2.5 R-Software

It is a free software programing language for statistical computing and graphics. This language is used for the generation of dendrograms which can be used for distance study. [8][9]

These dendrograms were generated with the help of parameters retrieved from the Protparam [6].

III. RESULTS AND DISCUSSION

3.1 Species of both plants and fungi showing homology with the enzymes present in the biosynthetic pathway of the alkaloid berberine found in plant *Berberis arsitata* are evident from BLAST result, are as follows**:-**

3.1.1 Enzyme 1

Berberine Bridge Enzyme (Homologs)

S.NO	SPECIES	ACCESSION NO.
1	*Eschscholzia californica*	P30986
2	*Argemone mexicana*	ACJ76783
3	*Coptis japonica*	BAM44344
4	*Thalictrum flavum subsp. glaucum*	AAU20769
5	*Berberis stolonifera*	AAD17487
6	*Papaver somniferum*	P93479

3.1.2 Enzyme 2

S-Adenosyl-L-Methionine (Homologs)

S.NO	SPECIES	ACCESSION NO.
1	*Zea mays*	NP_001169597
2	*Sorghum bicolor*	XP_002441466
3	*Setaria italic*	XP_004961340
4	*Oryza sativa Japonica Group*	BAH01482
5	*Oryza brachyantha*	XP_006655550
6	*Brachypodium distachyon*	XP_003567962
7	*Triticum*	AAL40895
8	*Aegilops*	EMT21187
9	*Triticum*	EMS54147
10	*Ostreococcus*	XP_003080348
11	*Micromonas*	XP_002499655
12	*Volvox*	XP_002947884
13	*Bathycoccus*	CCO14972

14	*Chlorella*	XP_005843906
15	*Chlamydomonas*	XP_001702380

3.1.3 Enzyme 3

Canadine Sythase (Homologs)

S.NO	SPECIES	ACCESSION NO.
1	*Thalictrum flavum*	ACO90244
2	*Thalictrum flavum subsp. glaucum*	AAU20771
3	*Coptis japonica*	Q948Y1
4	*Coptis japonica var. dissecta*	BAF98470
5	*Argemone mexicana*	B1NF19
6	*Papaver bracteatum*	ACO90234
7	*Eschscholzia californica*	BAG75116
8	*Papaver somniferum*	ADB89214

3.1.4 Enzyme 4

(S)-Tetrahydroberberine Oxidase (Homologs)

S.NO	SPECIES	ACCESSION NO.
1	*Coptis japonica*	BAJ40864
2	*Berberis wilsonae*	ADY15026
3	*Populus trichocarpa*	XP_002332196
4	*Vitis vinifera*	CAN81654
5	*Theobroma cacao*	EOY25686

6	*Citrus sinensis*	XP_006468345
7	*Citrus clementina*	XP_006448862
8	*Glycine max*	XP_003535119
9	*Arabidopsis thaliana*	NP_199254
10	*Capsella rubella*	XP_006280280
11	*Eutrema*	XP_006398055

3.1.5 Enzyme 5

(S)-Tetrahydroprotoberberine (Homologs)

S.NO	SPECIES	ACCESSION NO.
1	*Papaver somniferum*	P93479
2	*Argemone mexicana*	ACJ76783
3	*Eschscholzia californica*	P30986
4	*Berberis stolonifera*	AAD17487
5	*Thalictrum flavum subsp. glaucum*	AAU20769
6	*Coptis japonica*	BAM44344
7	*Phaseolus vulgaris*	ESW15912
8	*Glycine max*	XP_003555533

3.2 Physico-chemical properties, Computation of various physical and chemical parameters of the proteins present in Swissprot and trEMBL or a user entered sequence is done with the help of ProtParam. the computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathocity (GRAVY) are as follows:-

3.2.1 Enzyme 1 (Berberine bridge enzyme)

s.no	No.of amino acids	Molecular weight	PI	half-life (hours)	Instability index	Aliphatic index	Extinction coefficient (M^{-1} cm^{-1})		Hydropathocity (avg)
							residues form cys	Cys residues are reduced	
1	538	59958.3	5.27	30	35.42	90.24	94100	93850	-0.091
2	554	62533.4	5.40	30	32.86	91.12	101675	101300	-0.133
3	533	59261.5	5.53	30	33.85	88.69	94810	94310	-0.067
4	535	59734.5	5.77	30	37.02	92.02	90215	89840	-0.083
5	536	59760.2	5.15	30	34.90	86.23	98695	98320	-0.065
6	535	59903.3	5.30	30	34.97	84.17	94225	93850	-0.124

3.2.2 Enzyme 2 (s-adenosyl-l-methionine)

s.no	No. of amino acids	Molecular weight	PI	half-life (hours)	Instability index	Aliphatic index	Extinction coefficient (M^{-1} cm^{-1})		Hydropathocity (avg)
							residues form cys	Cys residues are reduced	
1	501	56774.5	6.02	30	37.85	80.30	69955	69330	-0.390
2	510	57652.5	5.69	30	38.29	81.92	65820	65320	-0.385
3	498	56707.3	5.76	30	36.84	78.25	74300	73800	-0.440
4	495	56451.0	5.86	30	38.68	77.39	74425	73800	-0.464
5	495	56273.8	6.24	30	38.50	76.61	7859	72310	-0.461
6	504	57263.0	6.43	30	38.39	78.13	74425	73800	-0.451
7	498	56858.3	5.21	30	38.27	79.62	74425	73800	-0.420

8	503	56844.3	5.41	30	39.23	80.83	71320	70820	-0.370
9	504	57047.4	5.25	30	39.63	80.48	71320	70820	-0.387
10	331	35139.8	6.97	30	37.93	84.71	24660	24410	-0.096
11	329	34439.2	6.46	30	38.76	84.41	23295	22920	0.007
12	335	35806.8	8.96	30	36.30	84.33	23295	22920	-0.099
13	321	33973.5	6.25	30	41.47	80.97	23170	22920	-0.081
14	263	28642.5	6.17	4.4	42.09	81.71	21680	21430	-0.238
15	326	34992.9	8.56	30	37.81	83.62	23170	22920	-0.126

3.2.3 Enzyme 3 (canadine synthase)

s.no	No. of amino acids	Molecular weight	PI	half-life (hours)	Instability index	Aliphatic index	Extinction coefficient (M^{-1} cm^{-1})		Hydropathocity (avg)
							residues form cys	Cys residues are reduced	
1	78	8641.1	7.73	1.9	43.09	78.97	5625	5500	0.187
2	492	55376.4	8.61	30	37.61	97.36	76110	75860	-0.005
3	491	55352.5	8.69	30	37.06	96.17	73590	73340	-0.053
4	491	55287.4	8.92	30	38.22	94.79	72100	71850	-0.054
5	504	57452.4	9.14	30	44.74	93.23	78965	78840	-0.217

6	198	22599.6	8.96	100	37.94	96.46	21095	20970	-0.067
7	495	56544.8	8.69	30	39.65	98.30	68090	67840	-0.092
8	494	55860.1	9.02	30	39.63	99.25	73130	72880	-0.085

3.2.4 Enzyme 4 ((s)-tetrahydroberberine oxidase)

s. no	No. of amino acids	Molecular weight	PI	half-life (hours)	Instability index	Aliphatic index	Extinction coefficient (M^{-1} cm^{-1})		Hydropathocity (avg)
							residues form cys	Cys residues are reduced	
1	540	60654.6	6.14	30	39.90	92.24	84020	84020	-0.122
2	530	58989.7	6.95	30	36.41	89.70	74175	73800	-0.059
3	527	59529.9	8.79	30	37.04	93.40	90550	90300	-0.089
4	539	60590.3	8.79	30	32.55	85.18	93530	93280	-0.112
5	546	61109.1	8.63	30	39.04	88.04	85175	84800	-0.144
6	535	59972.6	7.12	30	37.12	88.77	88030	87780	-0.144
7	542	60833.7	8.09	30	37.08	87.25	88030	87780	-0.165
8	543	61209.0	6.58	30	35.90	88.51	104990	104740	-0.222
9	535	61273.5	8.36	30	42.21	89.44	91010	90760	0.211
10	538	61444.5	8.63	30	40.88	86.54	92500	92250	-0.197
11	540	61470.5	8.97	30	37.33	88.07	88030	87780	-0.189

3.2.5 Enzyme 5 ((s)-tetrahydroprotoberberine)

s.no	No.of amino acids	Molecular weight	PI	half-life (hours)	Instability index	Aliphatic index	Extinction coefficient (M^{-1} cm^{-1})		Hydropathocity (avg)
							residues form cys	Cys residues are reduced	
1	535	59903.3	5.30	30	34.97	84.17	94225	93850	-0.124
2	554	62533.4	5.40	30	32.86	91.12	101675	101300	-0.133
3	538	59958.3	5.27	30	35.42	90.24	94100	93850	-0.091
4	536	59760.2	5.15	30	34.90	86.23	98695	98320	-0.065
5	535	59734.5	5.77	30	37.02	92.02	90215	89840	-0.083
6	533	59261.5	5.53	30	33.85	88.69	94810	94310	-0.067
7	550	61412.8	5.51	30	42.23	88.09	101230	100730	-0.121
8	550	61035.4	5.54	30	37.89	85.27	101355	100730	-0.060
9	550	61389.9	5.73	30	37.76	87.02	99865	99240	-0.095
10	259	28107.0	5.74	1.1	33.33	91.08	43680	43430	0.103
11	533	60023.4	8.88	30	43.11	82.83	79550	79300	-0.197
12	530	58810.1	7.11	30	31.02	90.55	92040	91790	-0.134
13	533	59758.8	9.65	30	36.10	87.11	95605	95230	-0.078

3.3 R is a language and environment for statistical computing and graphics. With the help of R language dendrograms are being formed of all the homologs evident by the BLAST results.[8][9] Here first a variable named enzyme1 is created to form a matrix from the physco-chemical parameters provided by the protparam. The matrix is filled by row. After this a list is created by the name dimnames. In this way matrix is formed followed by

the calculation of distance among the various species by the use of a function dist(distance). Which in the end generate a dendrogram which makes easier to identify the closest relationships among the species.

In the same way all the other parameters of enzymes are used in generation of dendrograms which helps us in distance study of the various plant and fungi species.

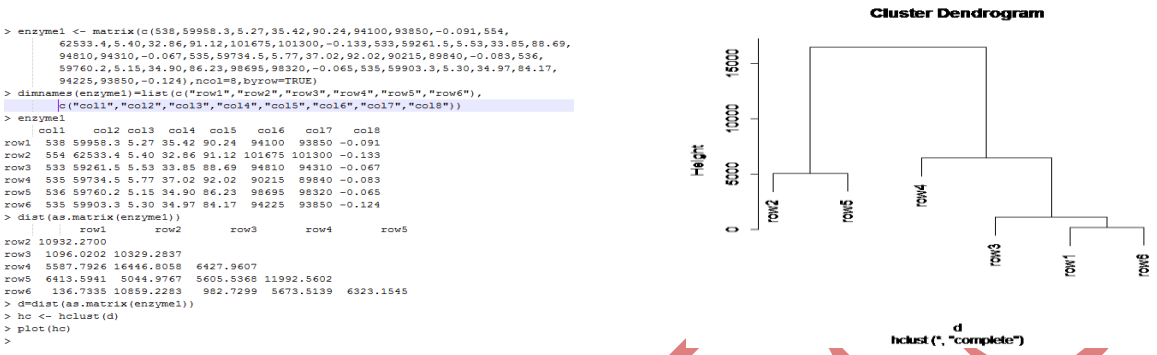

Fig 3. Dendogram for the Homologs of Enzyme 1

In comparison to all other branches, closest relationship is displayed between row1 *(Eschscholzia californica)*-row6 (*Papaver somniferum*) and row2 (*Argemone mexicana*)-row5 (*Berberis stolonifera*).

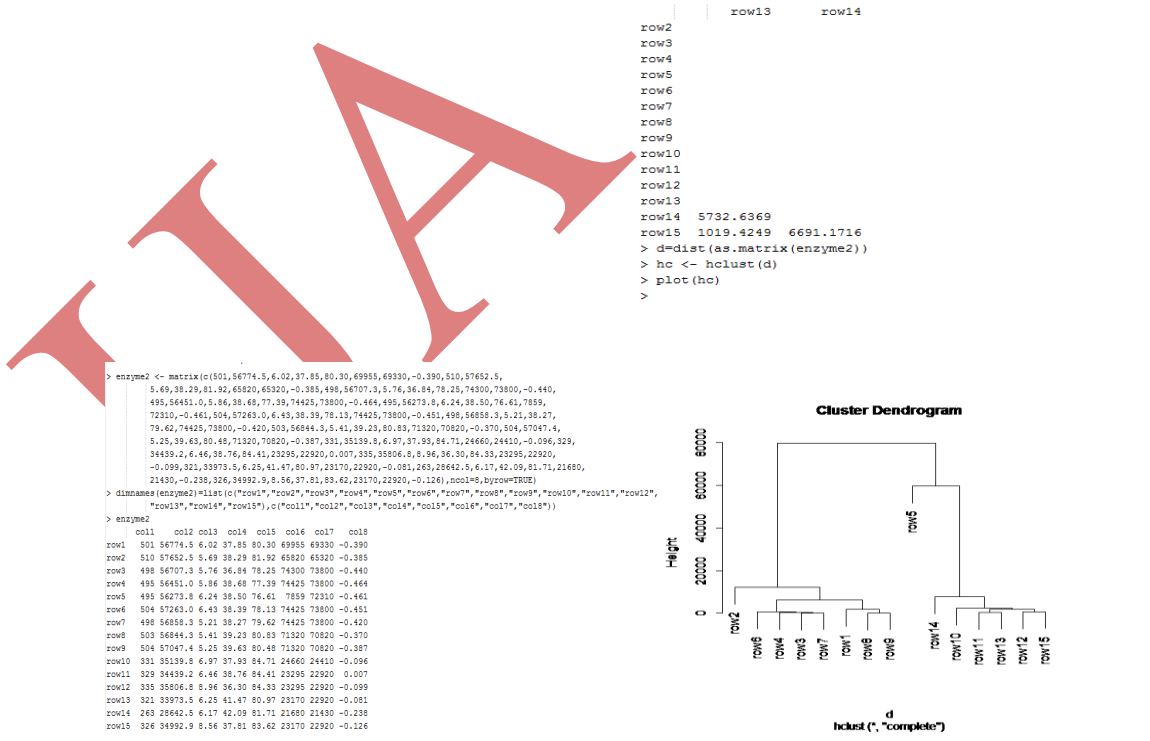

Fig4. Dendrogram for Enzyme 2

In comparison to other branches, closest relationship is displayed among

Row6 (*Brachypodium distachyon*), row4 (*Oryza sativa japonica group*), row3 (*Setaria italic*), row7 (*Triticum aseticum*).

Row11 (*Micromonas sp.*), row13 (*Bathycoccus prasinos*).

Row12 (*Volvox carteri f.nagariensis*), row15 (*Chlamydomonas reinhardtii)*

Row8 (*Aegilops tauschii*), row9 (*Triticum urartu)*

```
> enzyme3 <- matrix(c(78,8641.1,7.73,43.09,78.97,5625,5500,0.187,492,
   55376.4,8.61,37.61,97.36,76110,75860,-0.005,491,55352.5,8.69,37.06,96.17,
   73590,73340,-0.053,491,55287.4,8.92,38.22,94.79,72100,71850,-0.054,504,57452.4,
   9.14,44.74,93.23,78965,78840,-0.217,198,22599.6,8.96,37.94,96.46,21095,20970,-0.067,
   495,56544.8,8.69,39.65,98.30,68090,67840,-0.092,494,55860.1,9.02,39.63,99.25,
   73130,72880,-0.085),ncol=8,byrow=TRUE)
> dimnames(enzyme3)=list(c("row1","row2","row3","row4","row5","row6","row7","row8"),
   c("col1","col2","col3","col4","col5","col6","col7","col8"))
> enzyme3
      col1    col2 col3  col4  col5  col6  col7   col8
row1   78  8641.1 7.73 43.09 78.97  5625  5500  0.187
row2  492 55376.4 8.61 37.61 97.36 76110 75860 -0.005
row3  491 55352.5 8.69 37.06 96.17 73590 73340 -0.053
row4  491 55287.4 8.92 38.22 94.79 72100 71850 -0.054
row5  504 57452.4 9.14 44.74 93.23 78965 78840 -0.217
row6  198 22599.6 8.96 37.94 96.46 21095 20970 -0.067
row7  495 56544.8 8.69 39.65 98.30 68090 67840 -0.092
row8  494 55860.1 9.02 39.63 99.25 73130 72880 -0.085
> dist(as.matrix(enzyme3))
          row1      row2      row3      row4      row5      row6
row2 110013.7485
row3 106787.7925  3563.8987
row4 104867.9945  5671.6954  2108.1844
row5 114630.8680  4619.6767  7971.8531 10033.7313
row6  25951.8064 84344.3382 81062.8166 79112.9733 88953.2752
row7 100414.5934 11402.0162  7869.0283  5808.7249 15494.8216 74549.9279
row8 106427.4254  4242.0252   825.1563  1565.1889  8491.4314 80676.1291
```

```
> d=dist(as.matrix(enzyme3))
> hc <- hclust(d)
> plot(hc)
>
```

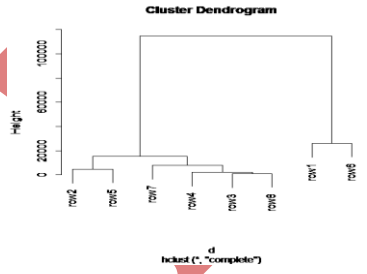

Fig5. Dendrogram for Enzyme 3

Closest homologies are-

Row2 (T*halictrum flavum subsp. glaucum*), row5 (*Argemon emexicana*)

Row3 (*Coptis japonica*), row8 (P*apaver somniferum*)

Row1 (*Thalictrum flavum*), row6 (*Papave rbracteatum*)

```
>  enzyme4 <- matrix(c(540,60654.6,6.14,39.90,92.24,84020,84020,-0.122,530,58989.7,
          6.95,36.41,89.70,74175,73800,-0.059,527,59529.9,8.79,37.04,93.40,90550,90300,-0.089,
          539,60590.3,8.79,32.55,85.18,93530,93280,-0.112,546,61109.1,8.63,39.04,88.04,85175,
          84800,-0.144,535,59972.6,7.12,37.12,88.77,88030,87780,-0.144,542,60833.7,8.09,37.08,
          87.25,88030,87780,-0.165,543,61209.0,6.58,35.90,88.51,104990,104740,-0.222,535,61273.5,
          8.36,42.21,89.44,91010,90760,0.211,538,61444.5,8.63,40.88,86.54,92500,92250,-0.197,540,
          61470.5,8.97,37.33,88.07,88030,87780,-0.189),ncol=8,byrow=TRUE)
> dimnames(enzyme4)=list(c("row1","row2","row3","row4","row5","row6","row7","row8","row9","row10","row11"),
          c("col1","col2","col3","col4","col5","col6","col7","col8"))
> enzyme4
      col1    col2 col3  col4  col5  col6   col7   col8
row1   540 60654.6 6.14 39.90 92.24 84020  84020 -0.122
row2   530 58989.7 6.95 36.41 89.70 74175  73800 -0.059
row3   527 59529.9 8.79 37.04 93.40 90550  90300 -0.089
row4   539 60590.3 8.79 32.55 85.18 93530  93280 -0.112
row5   546 61109.1 8.63 39.04 88.04 85175  84800 -0.144
row6   535 59972.6 7.12 37.12 88.77 88030  87780 -0.144
row7   542 60833.7 8.09 37.08 87.25 88030  87780 -0.165
row8   543 61209.0 6.58 35.90 88.51 104990 104740 -0.222
row9   535 61273.5 8.36 42.21 89.44 91010  90760  0.211
row10  538 61444.5 8.63 40.88 86.54 92500  92250 -0.197
row11  540 61470.5 8.97 37.33 88.07 88030  87780 -0.189
> dist(as.matrix(enzyme3))
> dist(as.matrix(enzyme4))
          row1      row2      row3      row4      row5      row6
row2  14287.9122
row3   9129.3174 23252.5798
row4  13273.7314 27507.2439  4345.7427
row5   1465.9658 15700.0677  7850.7892 11915.7743
row6   5539.2120 19707.0430  3591.2215  7802.6666  4280.5589
row7   5499.9834 19768.7104  3794.8594  7781.9842  4136.0961   861.1303
```

```
row8  29485.0250 43723.8298 20490.1650 16218.6939 28111.3473 24016.9097
row9   9729.8911 24005.7207  1861.0340  3628.7312  8342.4228  4410.5747
row10 11843.4491 26119.6146  3357.2125  1688.6474 10453.2653  6490.6333
row11  5557.2857 19838.2434  4057.9460  7827.8212  4142.7221  1497.9097
           row7       row8       row9      row10
row2
row3
row4
row5
row6
row7
row8  23987.9982
row9   4237.2520 19770.8135
row10  6350.9769 17665.0989  2114.1098
row11   636.8043 23986.4878  4218.9644  6321.5896
> d=dist(as.matrix(enzyme4))
> hc<-hclust(d)
> plot(hc)
>
```

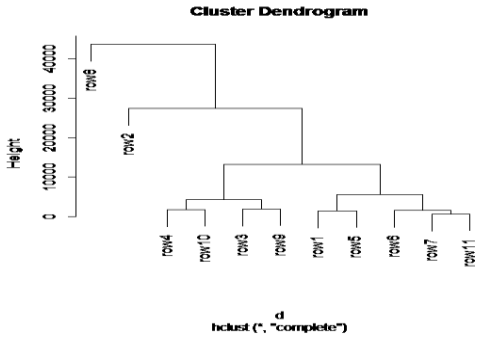

Fig7. Dendrogram for Enzyme 4

Closest homologs are-

Row4 (*Vitis vinifera*),row10 (*Capsella rubella*)

Row3 (*Populus trichocarpa*), row9 (*Arabidopsis thaliana*)

Row1 (*Coptis japonica*), row5 (*Theobromacacoa*)

Row7 (*Citrus clementina*), row11 (*Eutrema salsugineum*)

```
row5   5673.5139 16446.8058  5587.7926 11992.5602
row6    982.7299 10329.2837  1096.0202  5605.5368  6427.9607
row7   9933.9473  1333.7100 10014.3430  3868.5482 15580.0925  9330.6619
row8   9972.6218  1634.4355 10056.3286  3809.2048 15632.7960  9338.1188
row9   7941.7832  2971.0855  8021.0326  2207.1310 13572.8810  7374.8436
row10 78154.0424 88868.5601 78095.6846 83914.0283 72936.7484 78573.0822
row11 20665.7357 31302.0183 20576.9140 26988.1604 14997.2472 21418.4108
row12  3195.7778 14040.5438  3131.3975  9371.9095  2826.2529  3771.8808
row13  1956.9653  9021.5681  2051.6529  4369.9237  7622.6527  1313.6806
           row7        row8        row9       row10       row11       row12
row2
row3
row4
row5
row6
row7
row8    397.5960
row9   2020.8589  2136.7904
row10 87776.1561 87715.7904 85903.1226
row11 30515.5380 30589.6617 28498.6051 59933.6770
row12 13082.5946 13101.3458 11108.0580 74967.5463 17705.1550
row13  8039.0724  8058.6922  6073.5899 79883.3974 22618.5324  5044.0965
> d=dist(as.matrix(enzyme5))
> hc <- hclust(d)
> plot(hc)
>
```

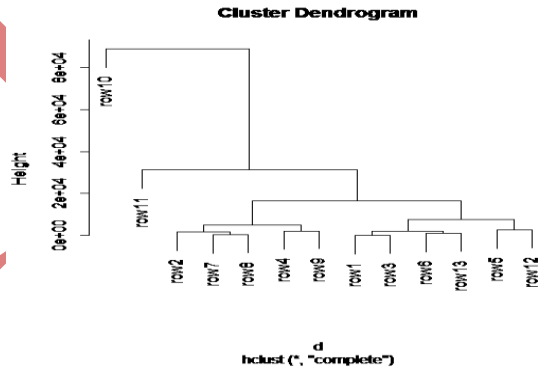

Fig7. Dendrogram for Enzyme 5

Closest homologs displayed by the above result are-

Row7 (*Phaseolus vulgaris*), row8 (*Glycine max*)

Row4 (*Berberis stolonifera*), row9 (*Vitisvinifera*)

Row1 (*Papavar somniferum*), row3 (*Eschscolzia california*)

Row6 (*Coptis japonica*), row13 (*Citrus sinensis*)

Row5 (*Thalictrum flavum*), row12 (*Theobroma cacao*)

The various results generated through public domain softwares showed that for enzyme 1 (Berberine bridge enzyme) involved in biosynthesis of Berberine 6 homologs were found in plant species.

Similarly for enzyme 2 (s-adenosyl-l-methionine) 15 homologs were seen in plants.

For enzyme 3 (canadine synthase) 8 homologs in plants.

For enzyme 4 ((s)-tetrahydroberberine oxidase) 11 homologs in plants.
For enzyme 5 ((s)-tetrahydroprotoberberine) 13 homologs in plants.

From all the above results we found that *Coptis japonica* is the most closest alternative to the alkaloid berberine present in the plant *Berberis aristata*. As the enymes present in the biosynthetic pathway of the alkaloid berberine were five out of which four of the enzymes were found in the plant *Coptis japonica.*

IV. CONCLUSION

Hence, we can say in-silico bioprospecting can be a very effective tool for finding alternative plant species or fungi species or bacterial species having the same enzyme which is been used widely by us. Thereby saving the plant species from getting endangered. This technique can be used for other plants or fungi species inorder to avoid their indiscriminate cutting and usage. Also in-silico techniques can prove to be cost and time effective. These provide higher precision and better quality of experimental data and provides instant access to numerable sets of data generated by scientific communities. More research can be done in this field which will lead to betterment of the world.

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