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SU et al.

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(54) **ISOLATED OR ENGINEERED POLYPEPTIDES, MICROORGANISMS AS WELL AS METHOD FOR SYNTHESIZING POLYPHENOLIC PHYTOCHEMICALS PHOSPHATE DERIVATIVE USING THE POLYPEPTIDES OR MICROORGANISMS**

(71) Applicant: **National Taiwan University**, Taipei (TW)

(72) Inventors: **NAN-WEI SU**, Taipei (TW); **CHEN HSU**, Taipei (TW)

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**CI2P 9/00** (2006.01)

**CI2P 17/06** (2006.01)

**CI2P 17/18** (2006.01)

(52) **U.S. Cl.**  
CPC ..... **CI2N 9/1294** (2013.01); **CI2P 9/00** (2013.01); **CI2P 17/06** (2013.01); **CI2P 17/181** (2013.01)

(57) **ABSTRACT**

The present invention provides an isolated or engineered polypeptide, a microorganism comprising a nucleic acid sequence encoded by the polypeptide, and a method for synthesizing a polyphenolic phytochemicals phosphate derivative using the polypeptide or the microorganism. The polypeptide having a homologous protein sequence that is more than 70% identical to the polyphenol phosphorylation synthetase (SEQ ID NO: 13) comprises a conserved domain which sequentially comprises: an ATP-binding domain, which includes active catalytic sites of Lys27, Arg102, and Glu282; a substrate-binding domain, which includes a conserved motif of DDHHFYIDAMLDKAR (SEQ ID NO: 14), and includes active catalytic sites of Asp627, His629, and His630; and a phosphorylated histidine catalytic domain, which includes His795 based on SEQ ID NO: 13.

**Specification includes a Sequence Listing.**

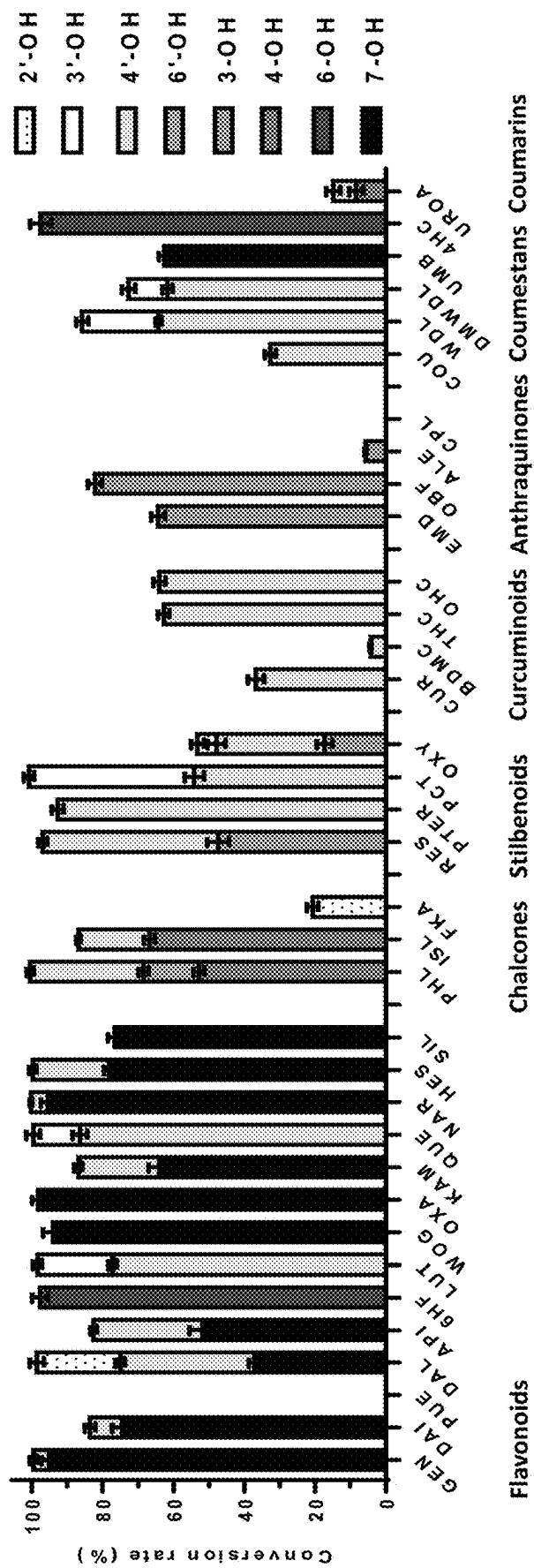


Figure 1

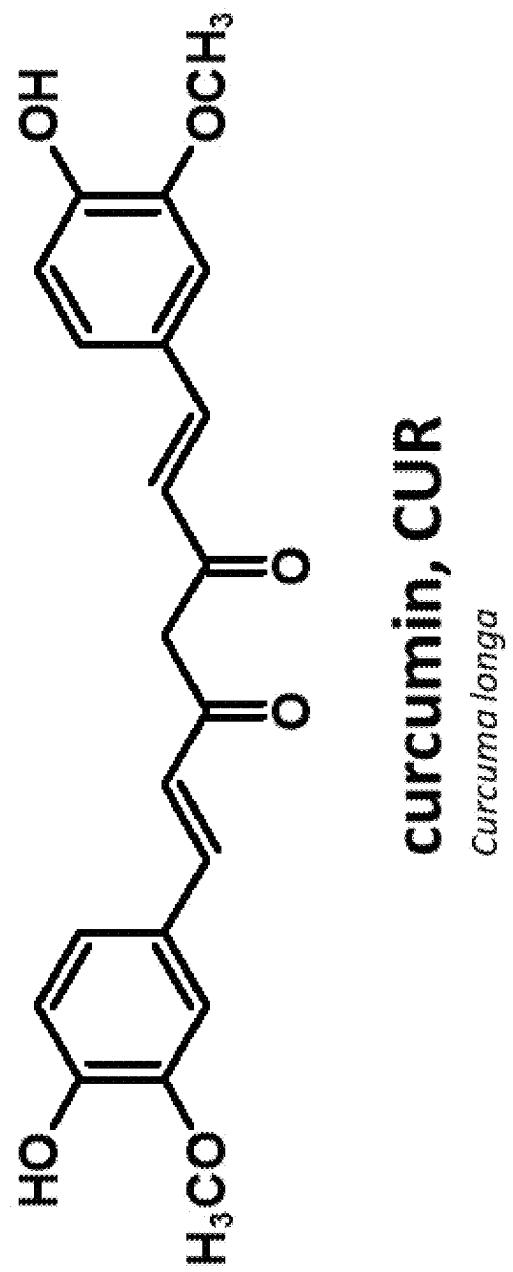


Figure.2(A)

Figure.2(B)

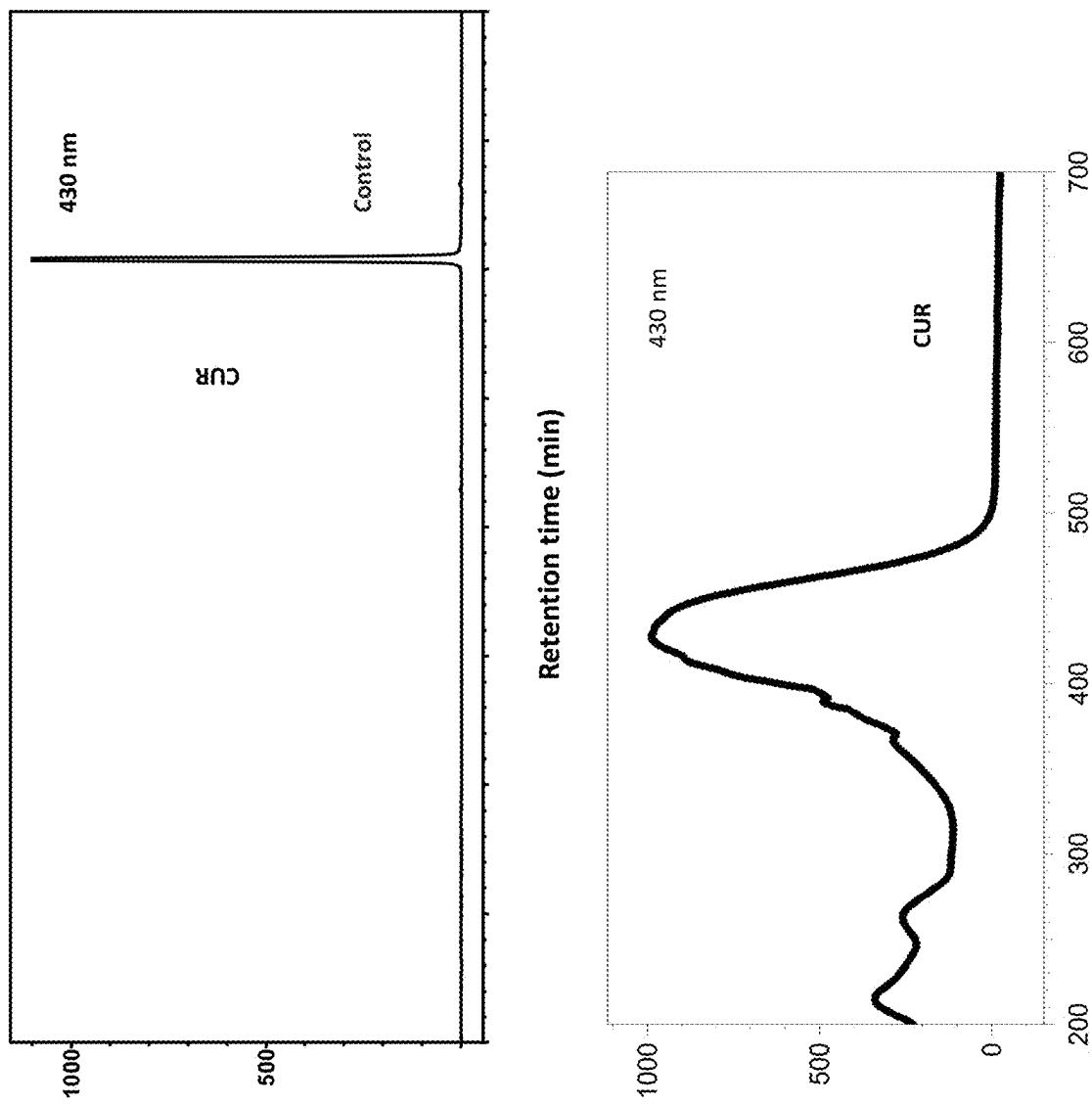
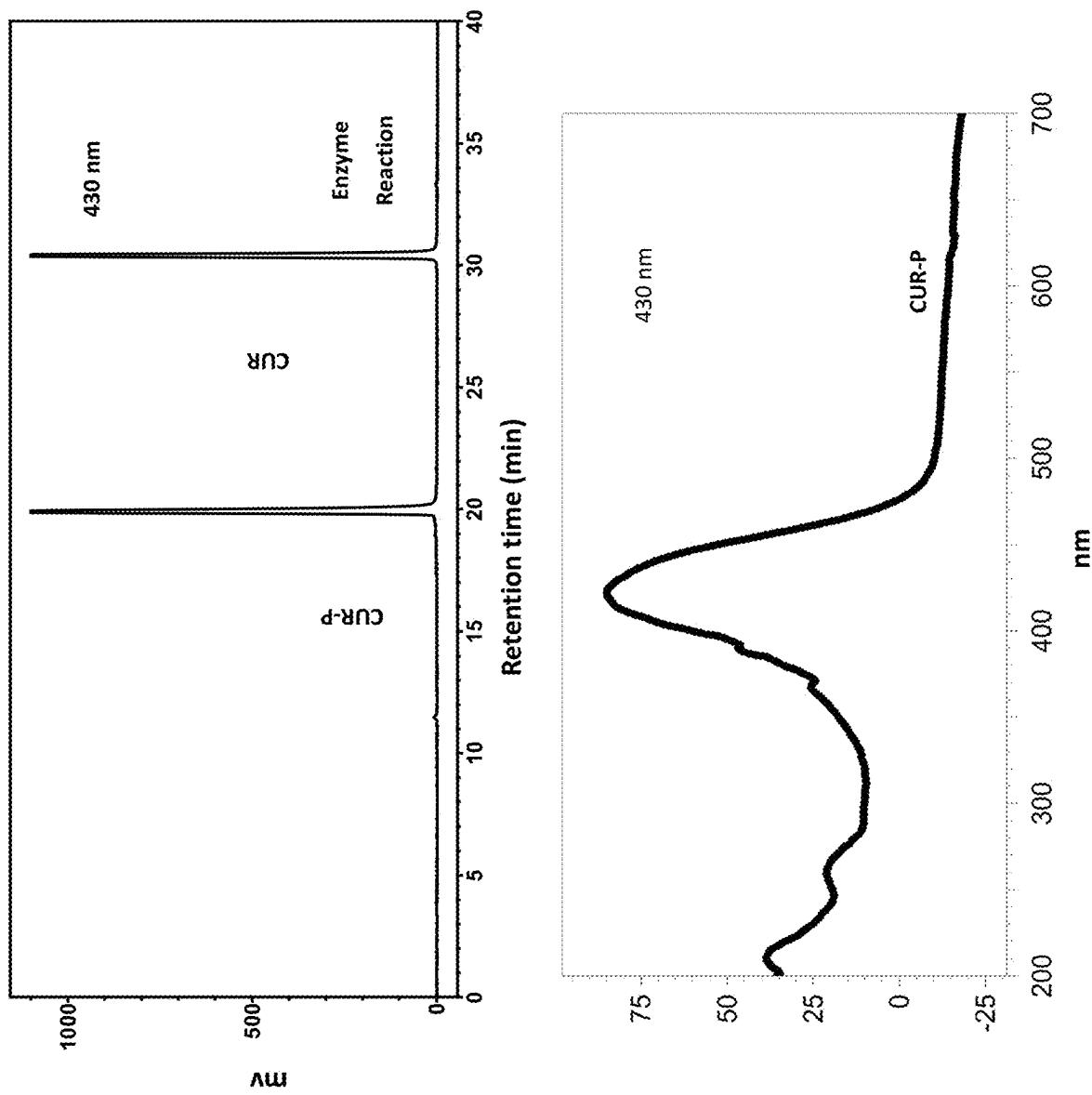


Figure.2(C)



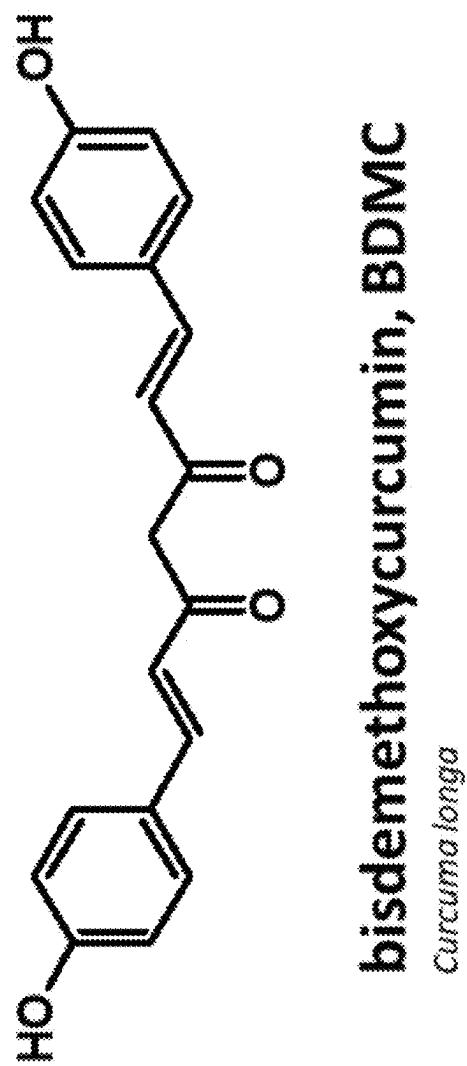


Figure 3(A)

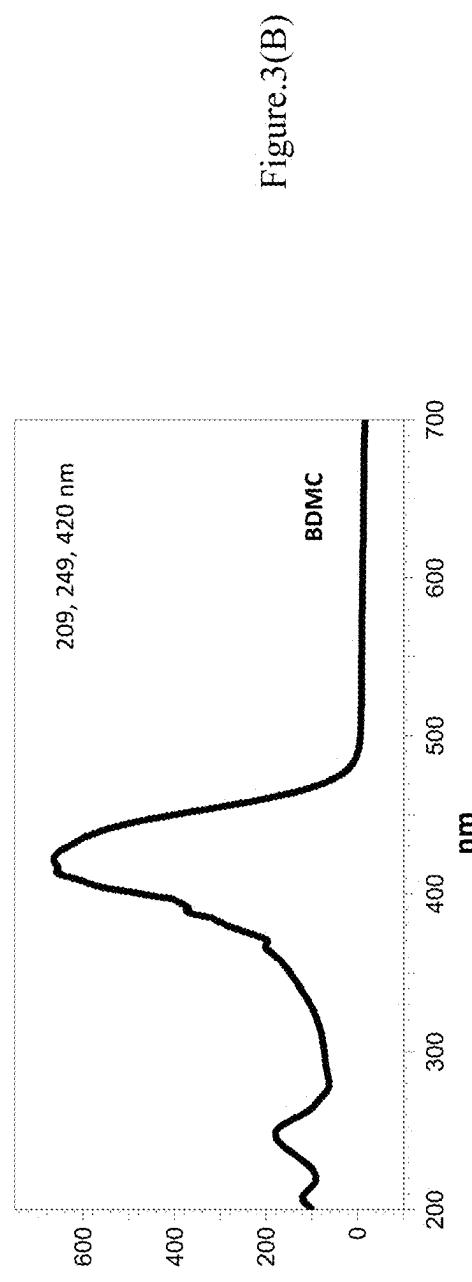
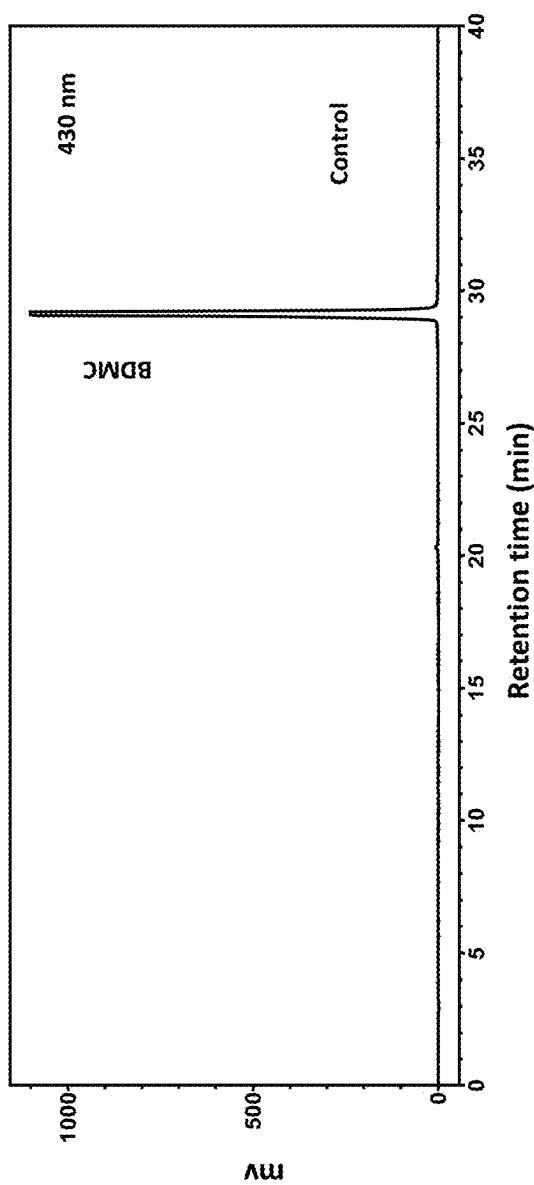


Figure.3(B)

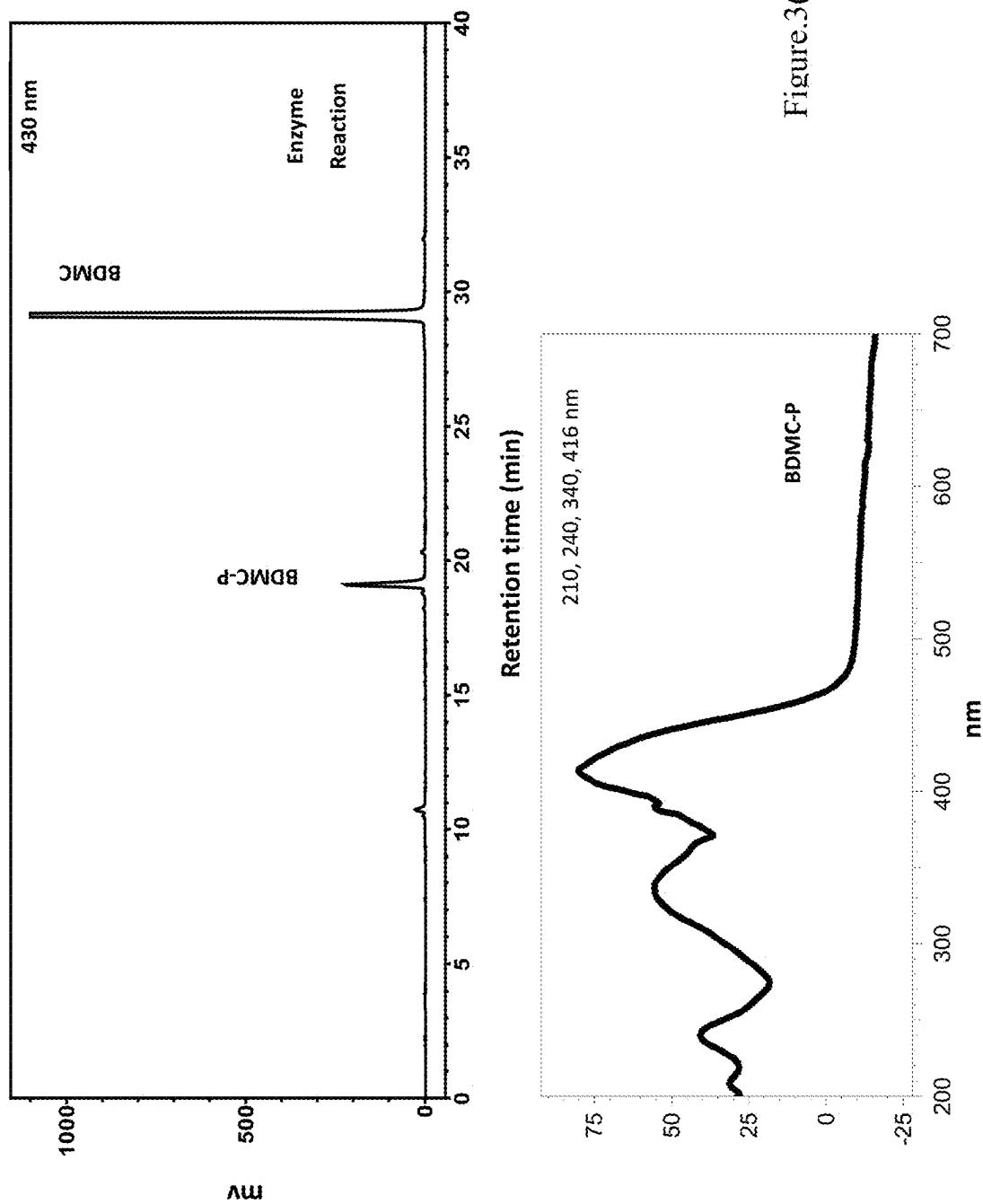
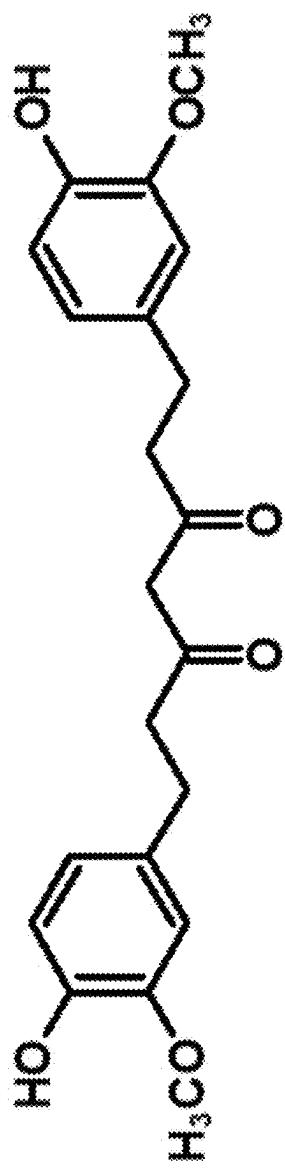


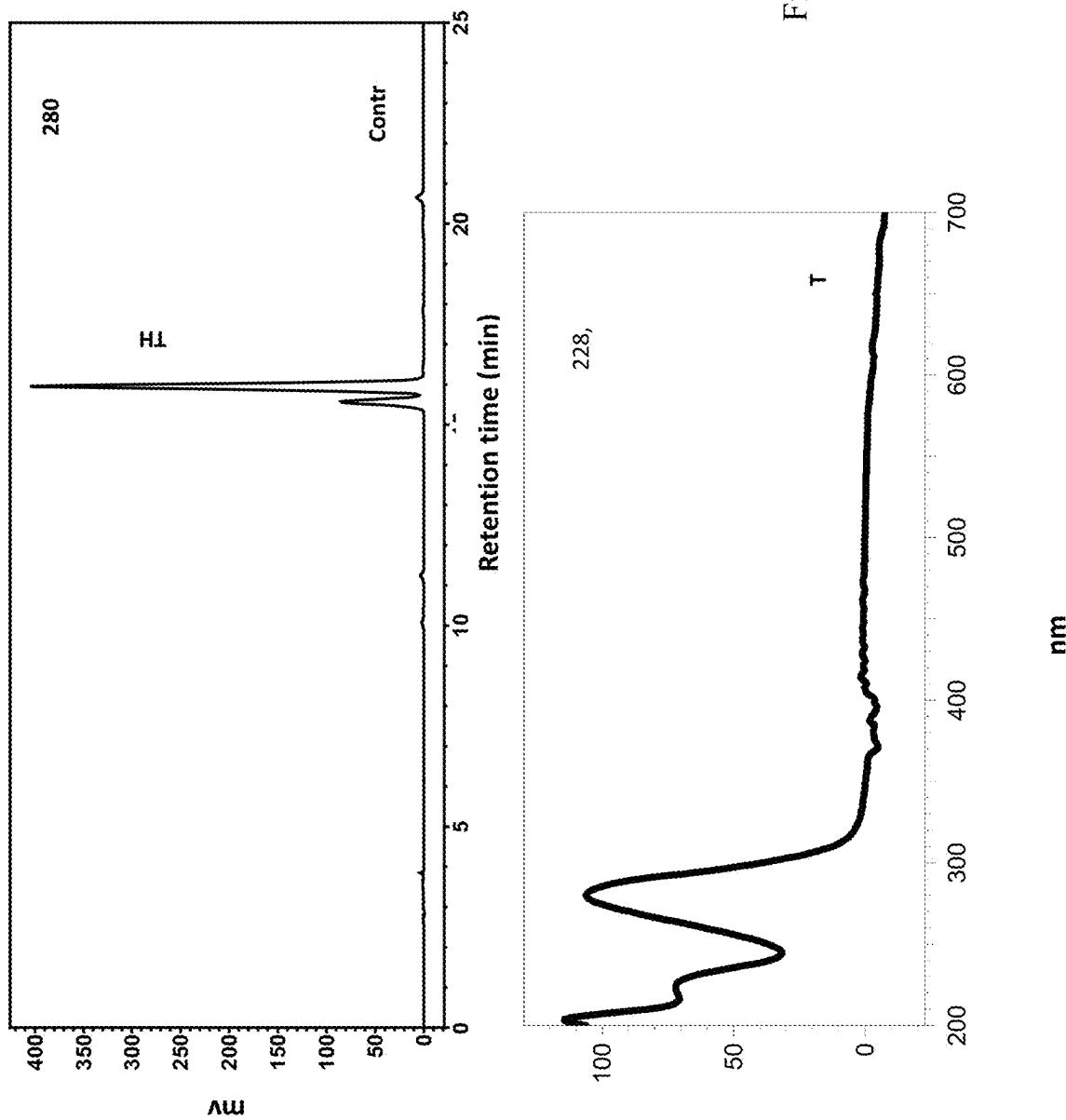
Figure.3(C)



tetrahydrocurcumin, THC

Curcumin metabolites

Figure 4(A)



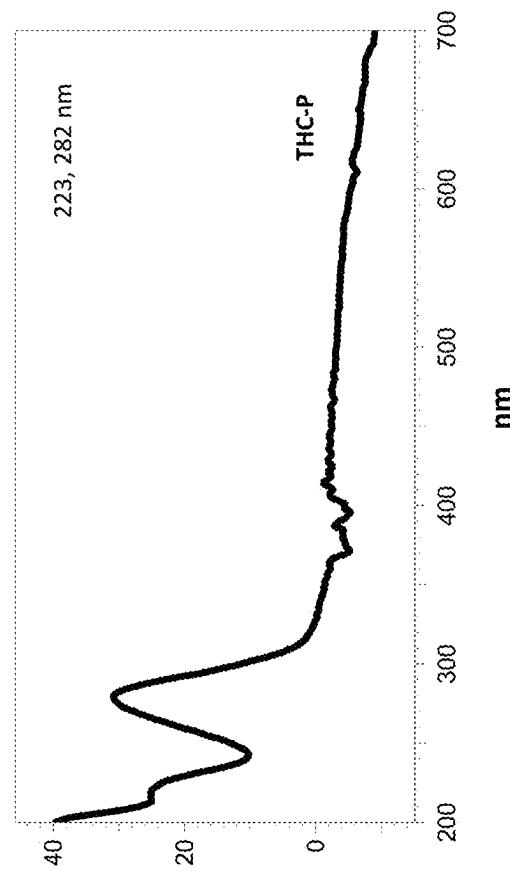
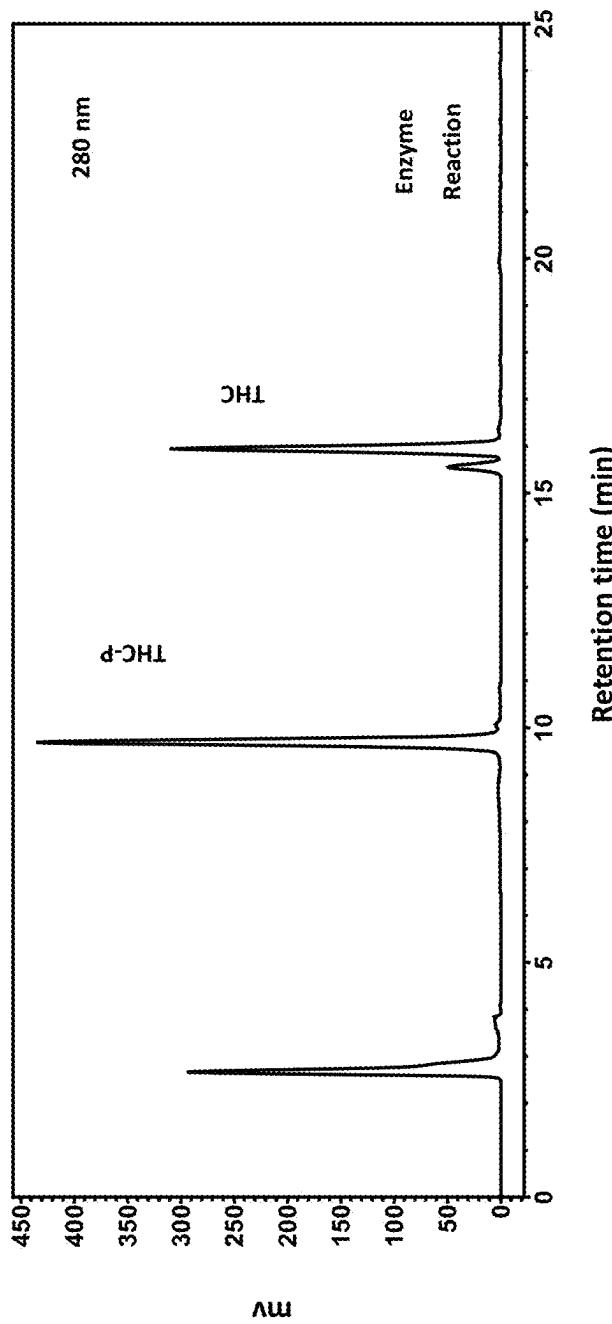
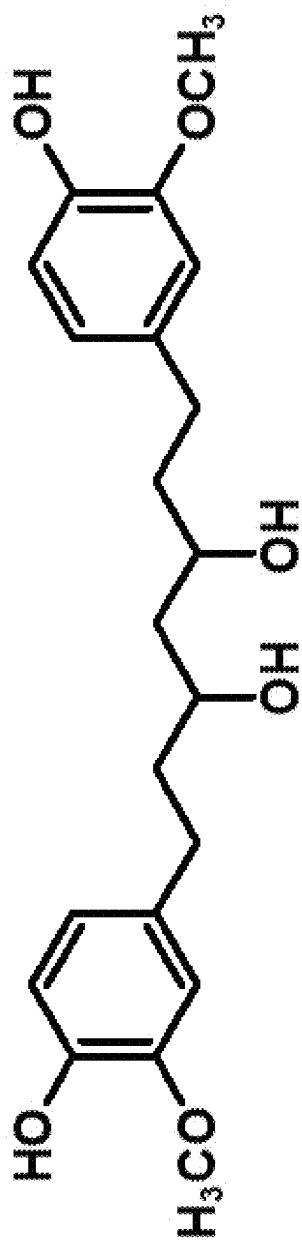


Figure 4(C)



cctahydrocurcumin, OHC

Curcumin metabolites

Figure 5(A)

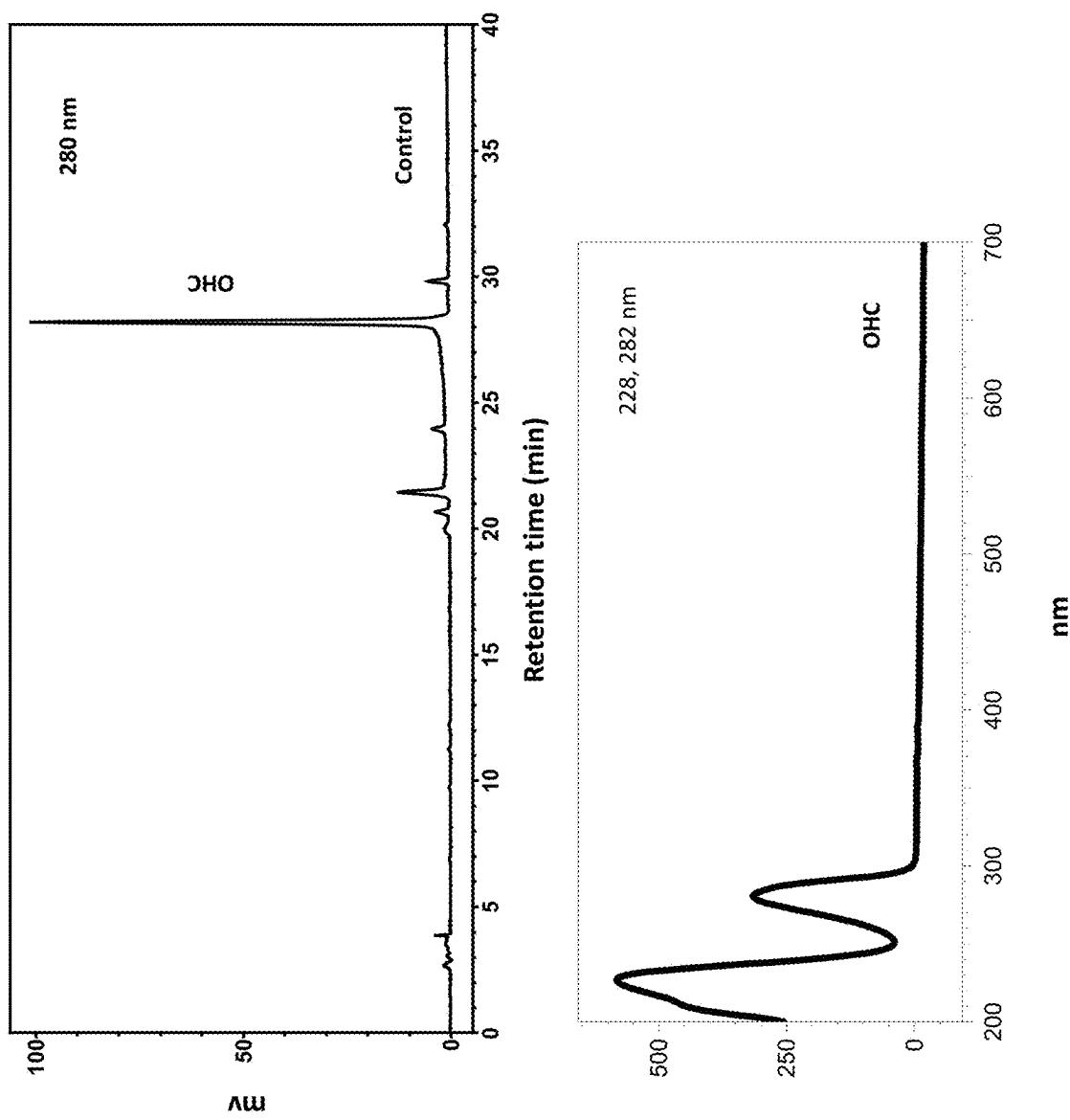


Figure.5(B)

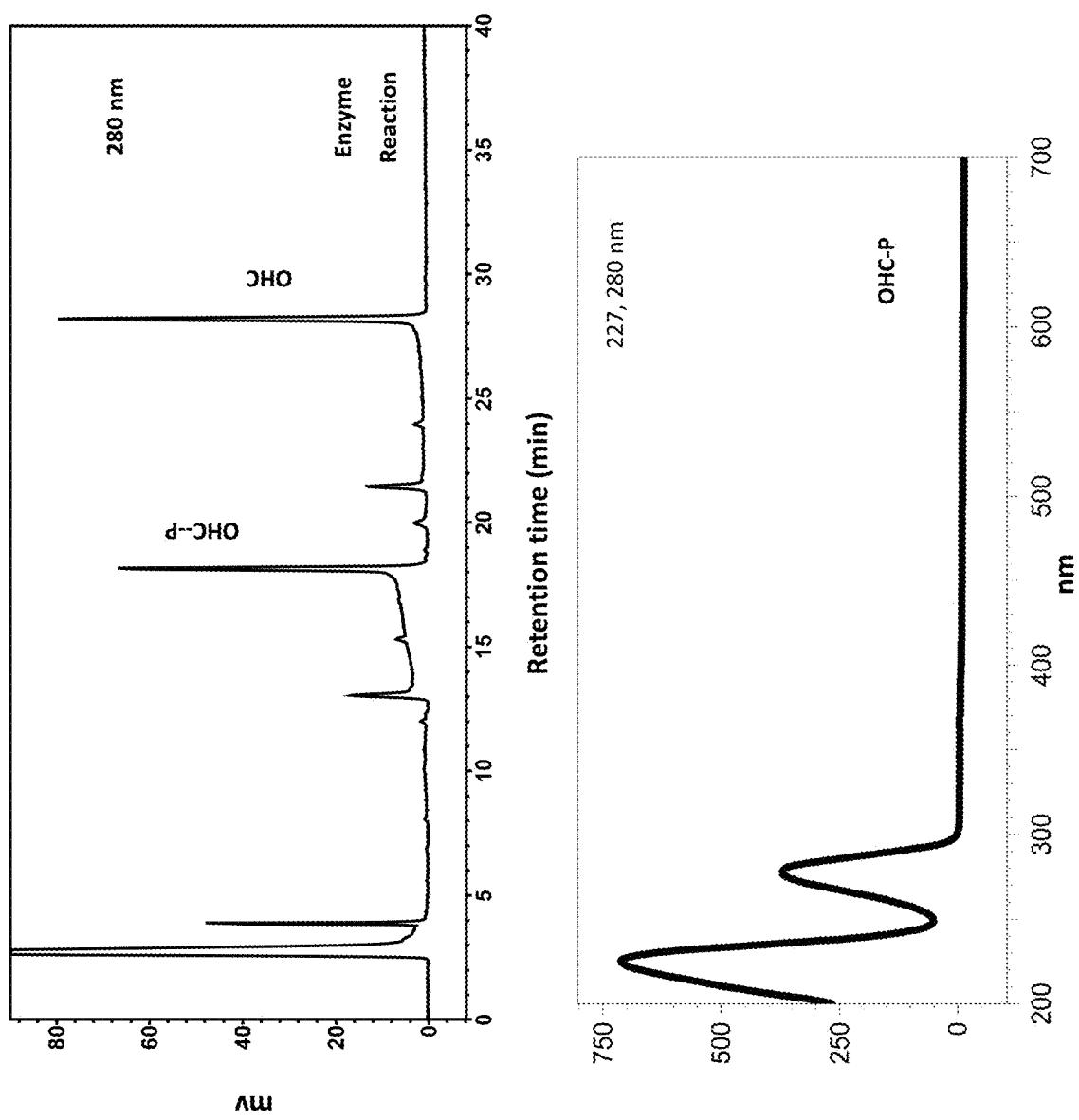
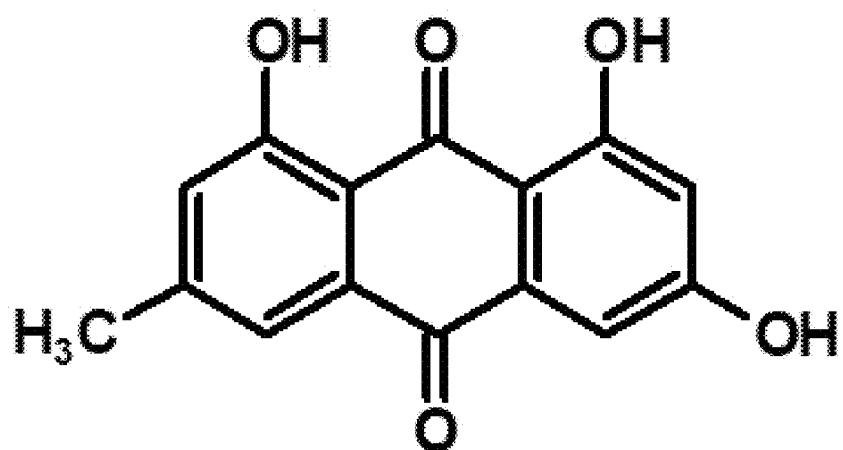


Figure.5(C)



## emodin, EMD

*Rheum rhabarbarum*

Figure.6(A)

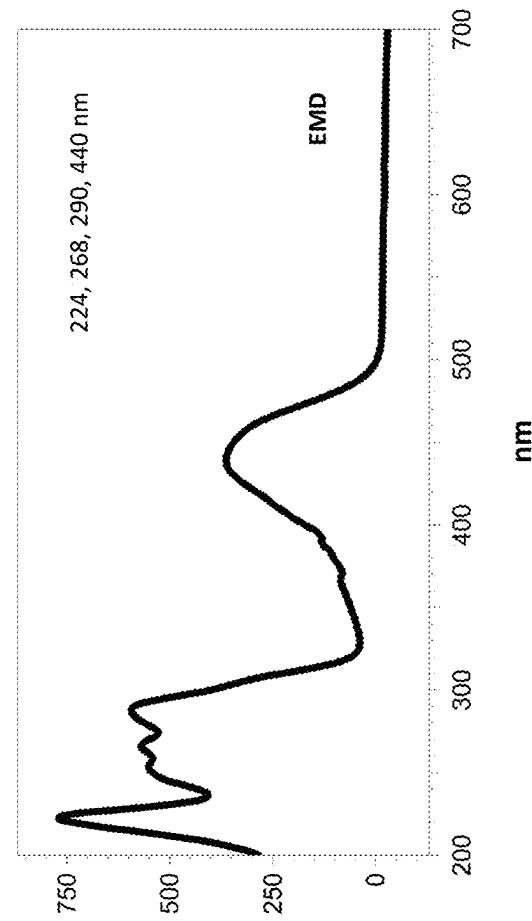
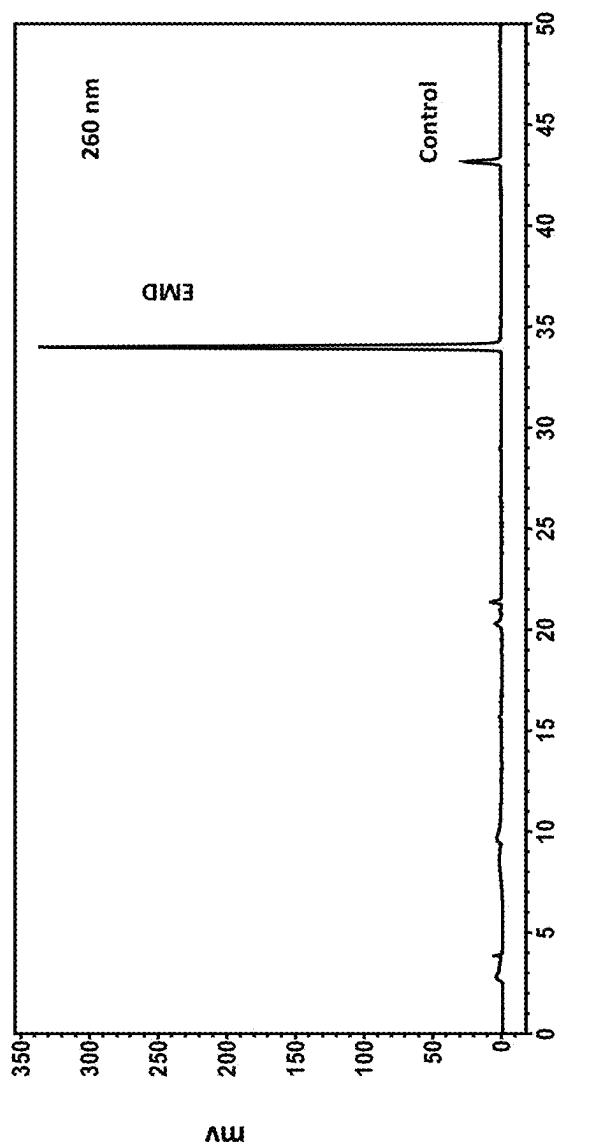


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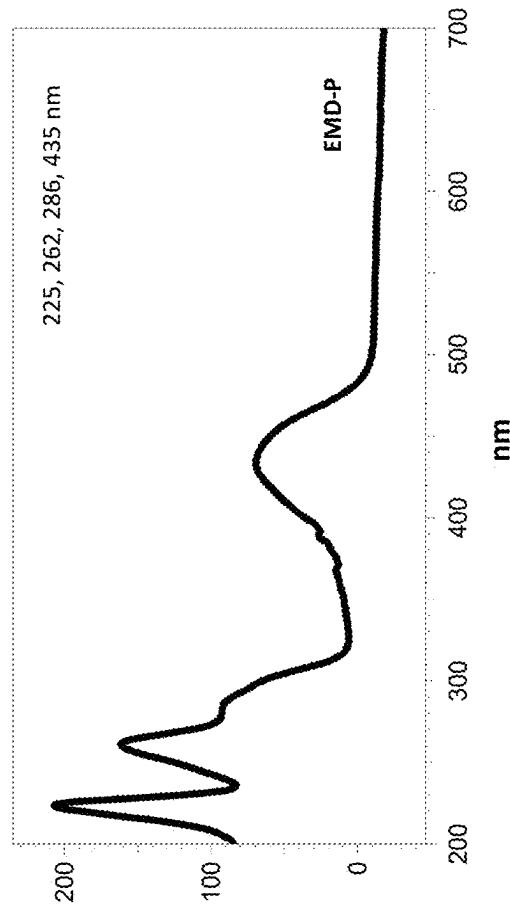
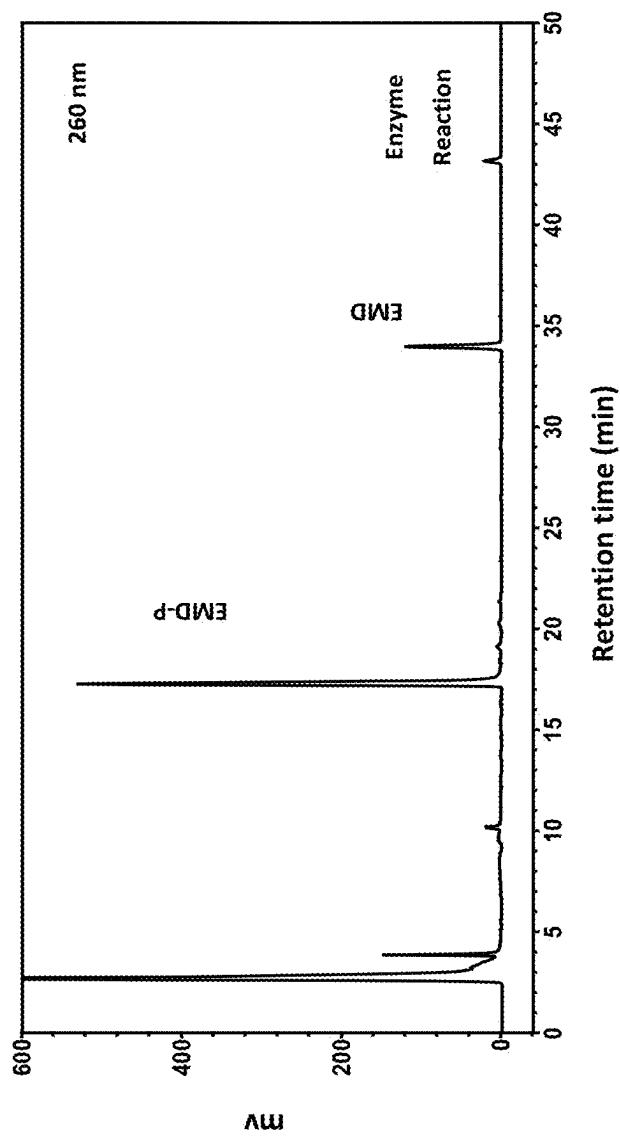
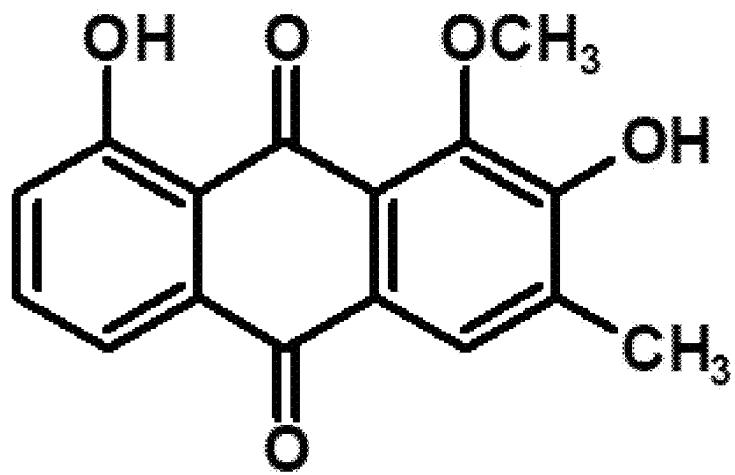


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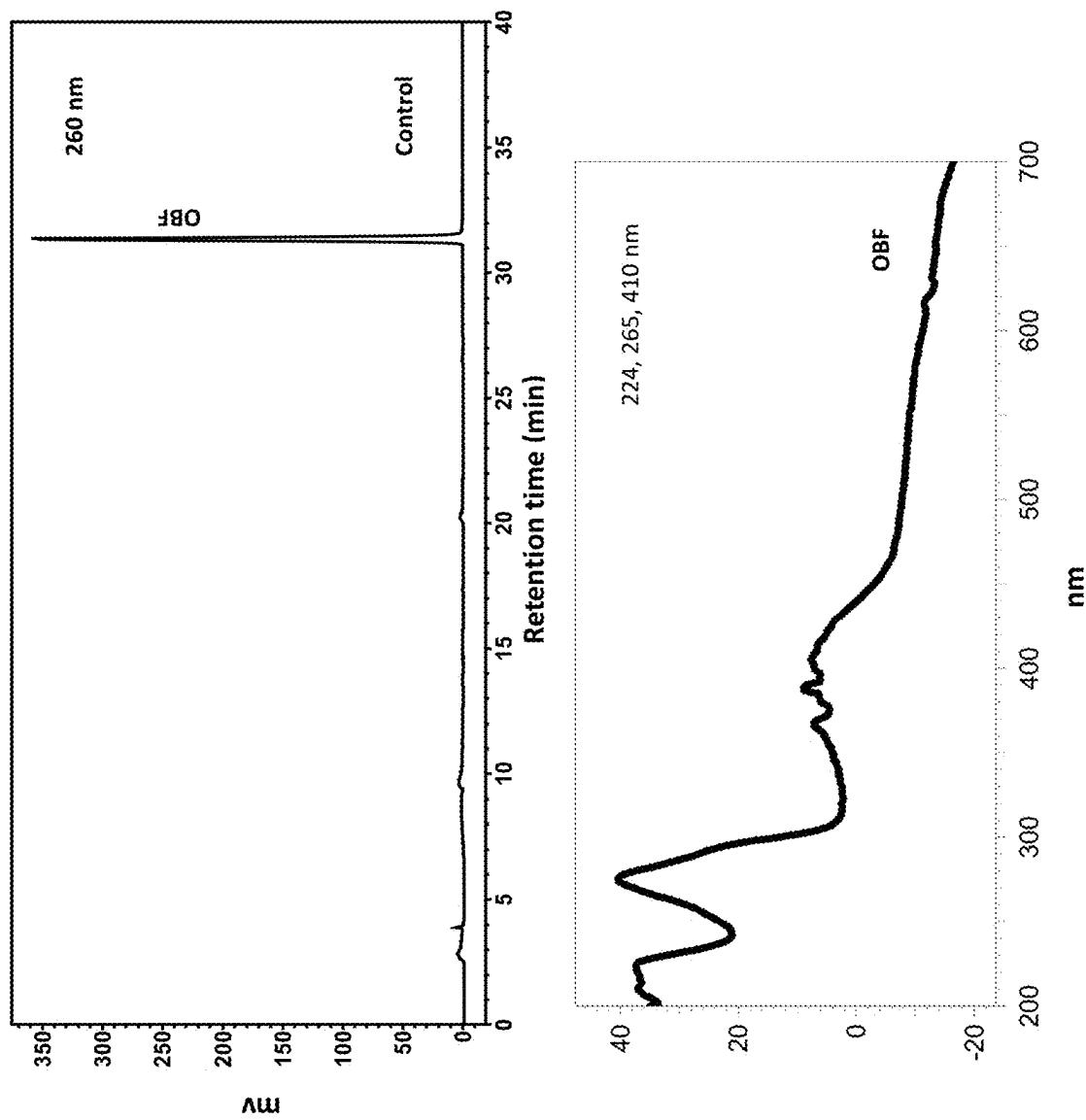


## **obtusifolin, OBF**

*Cassia obtusifolia*

Figure.7(A)

Figure.7(B)



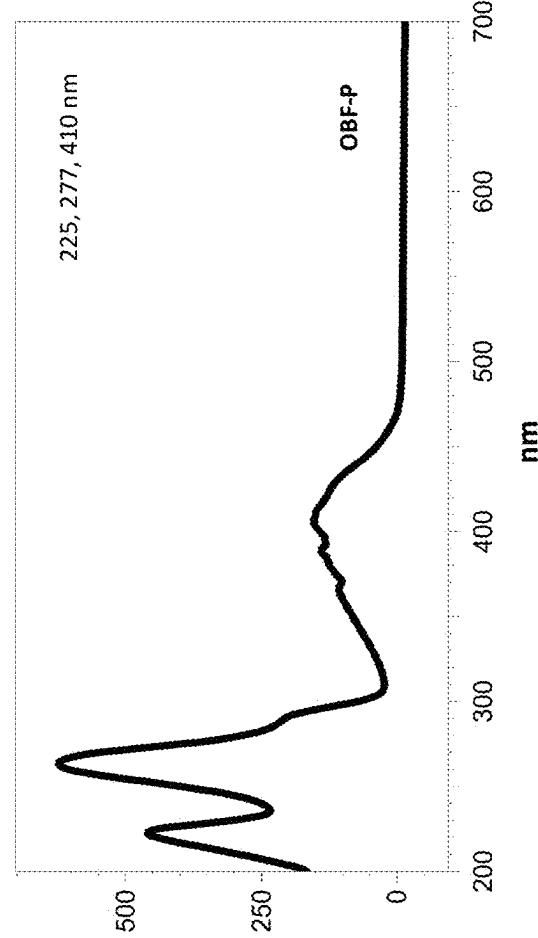
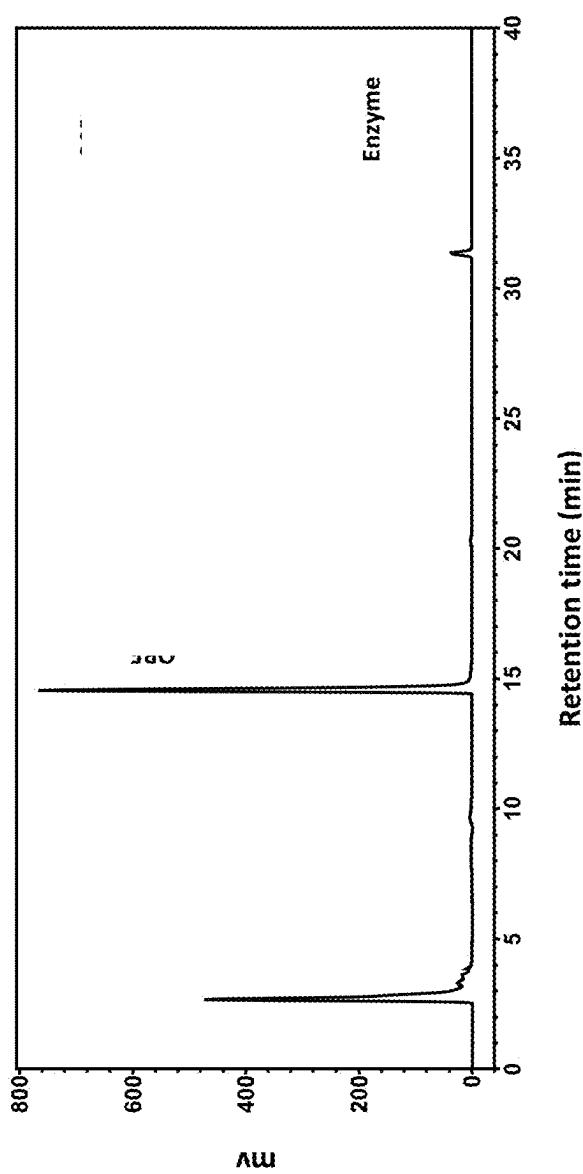
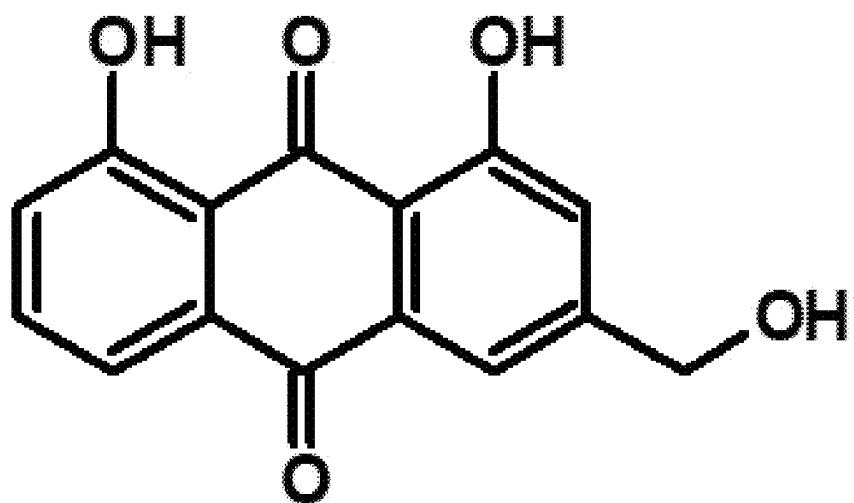


Figure.7(C)



## aloë-emodin, ALE

*Aloe*

Figure.8(A)

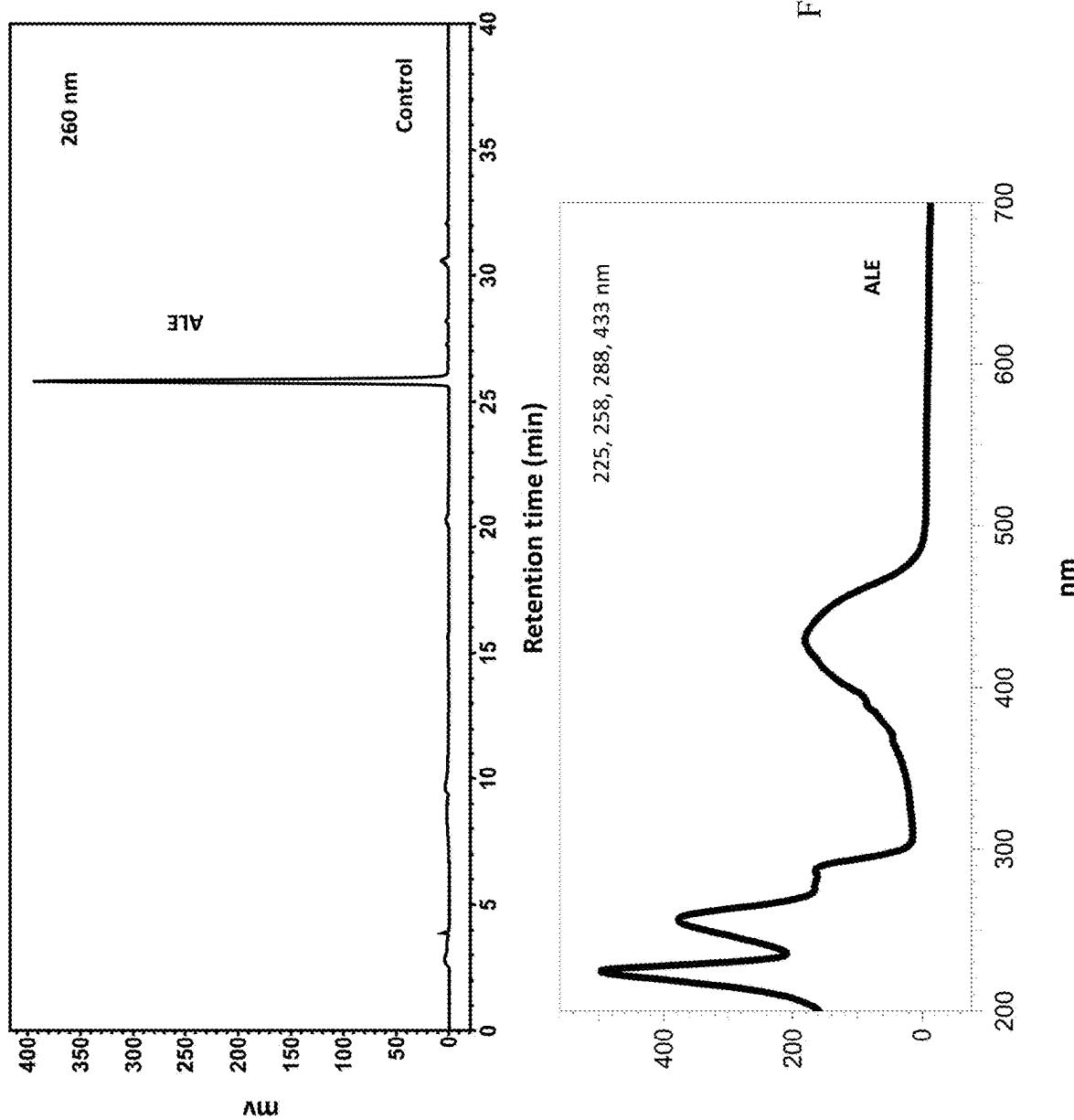


Figure.8(B)

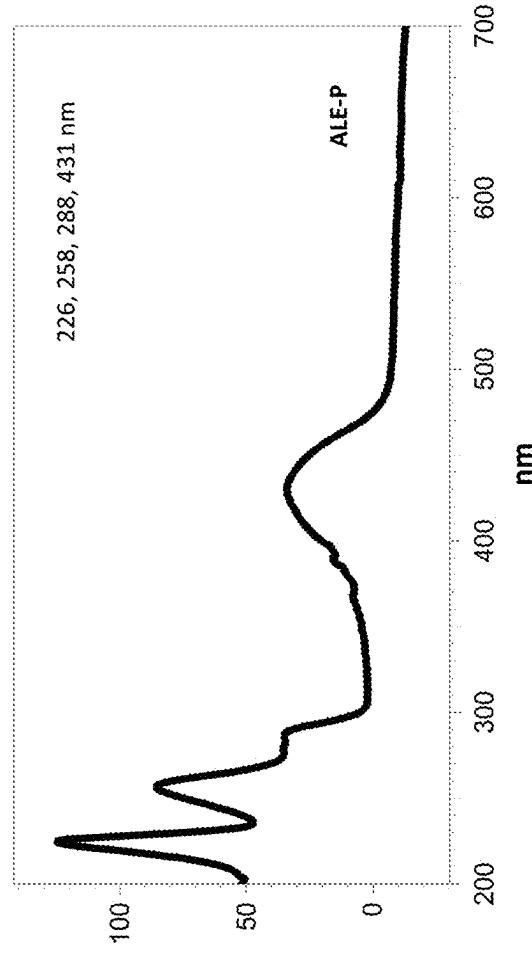
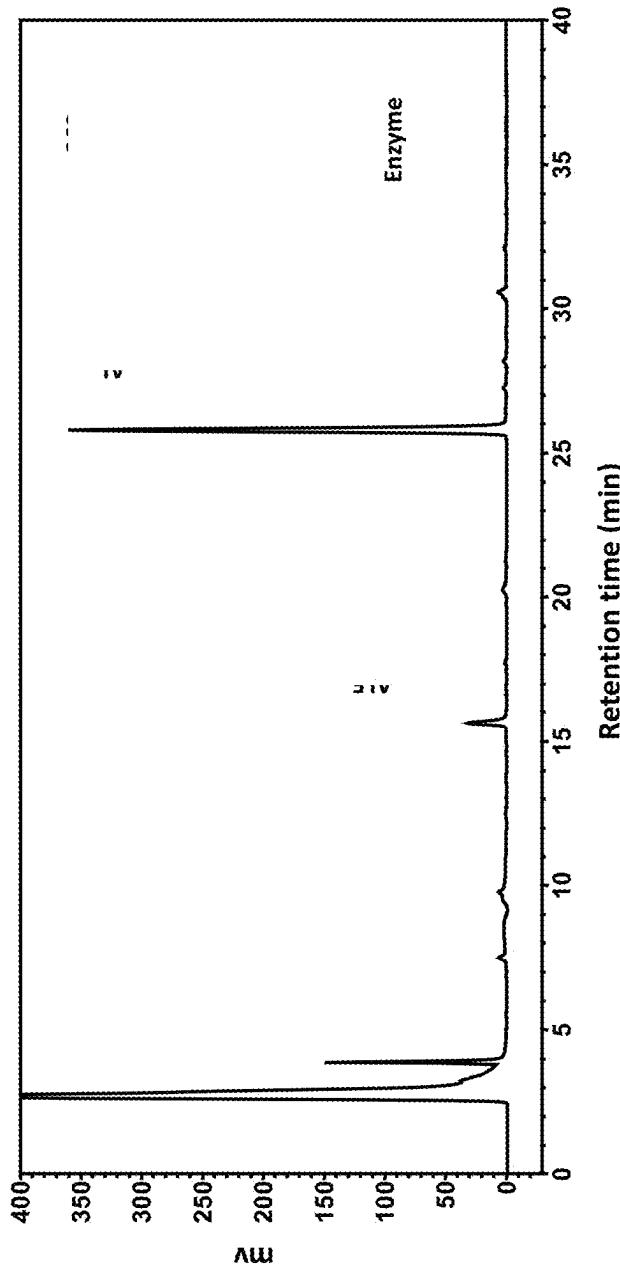
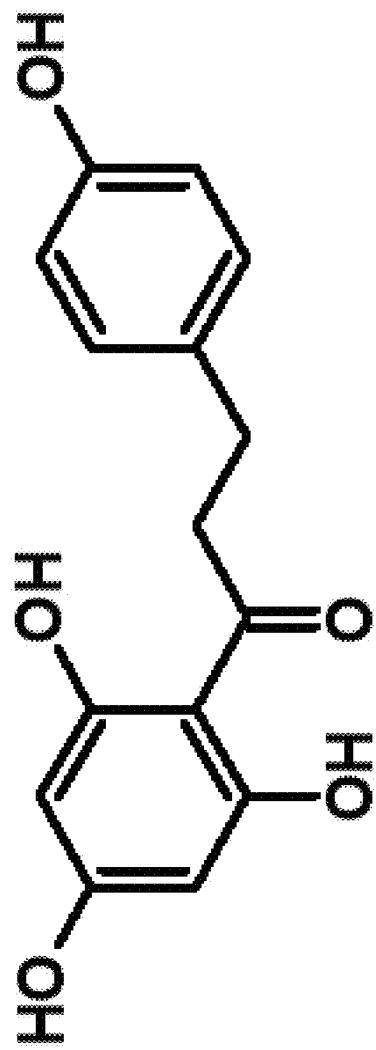


Figure.8(C)



**phloretin, PHL**  
*Prunus mandshurica*

Figure.9(A)

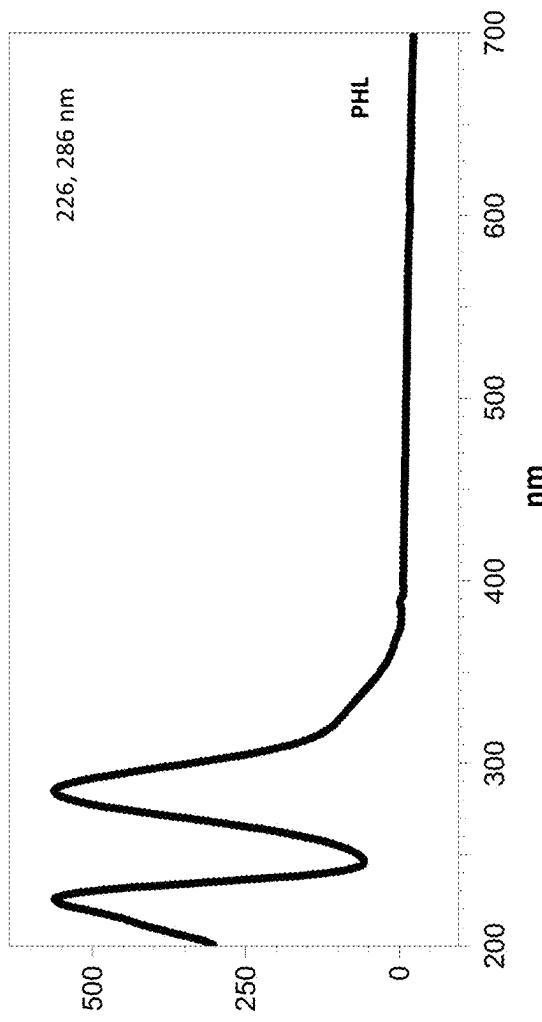
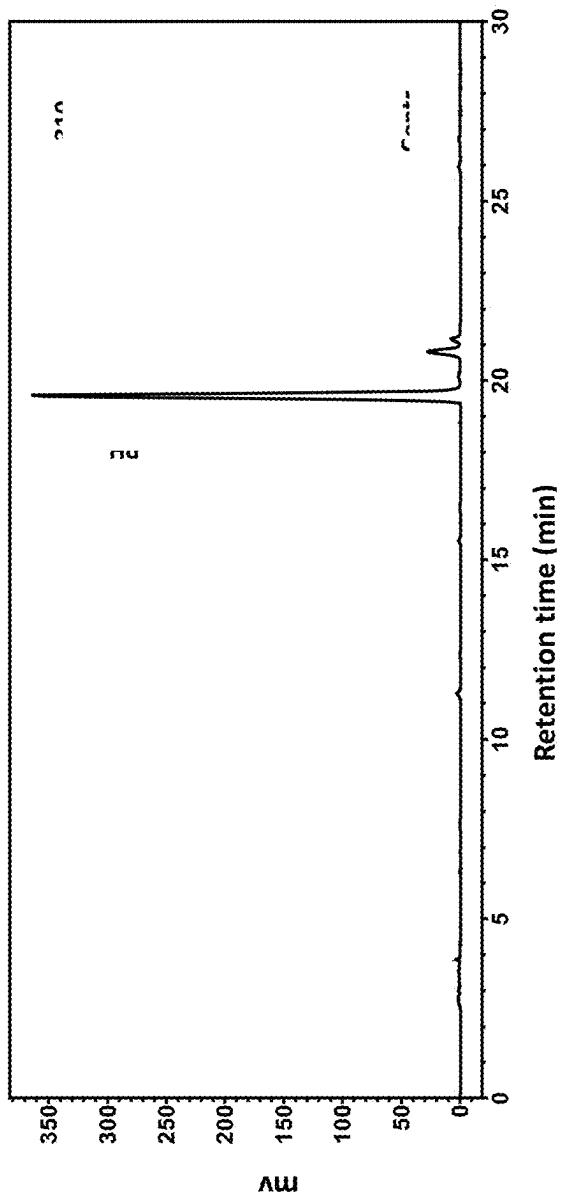


Figure.9(B)

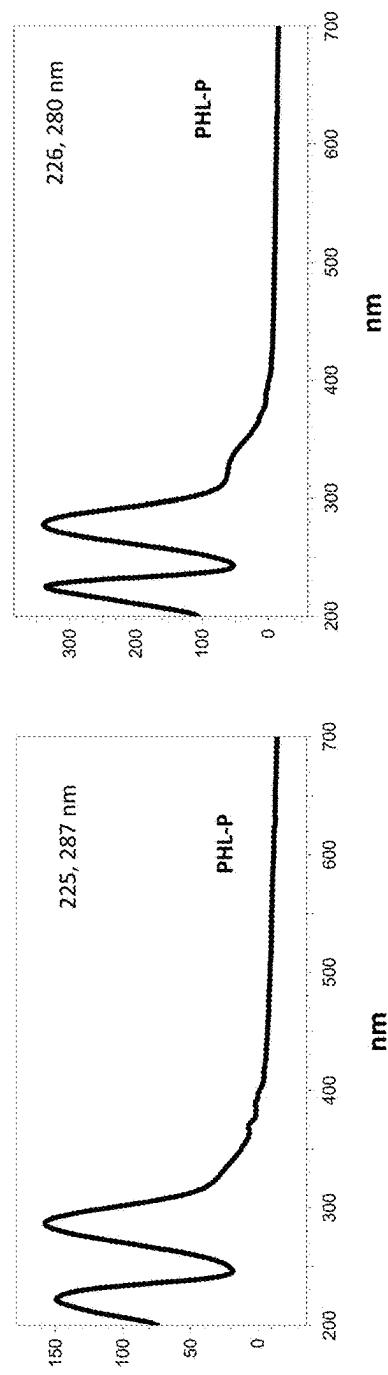
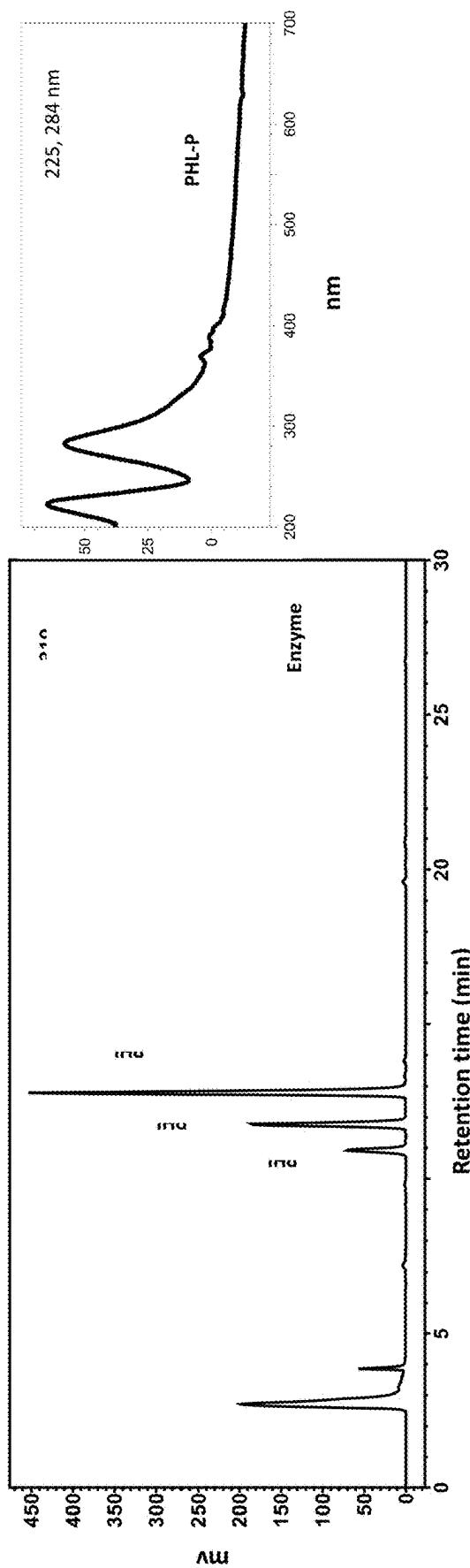
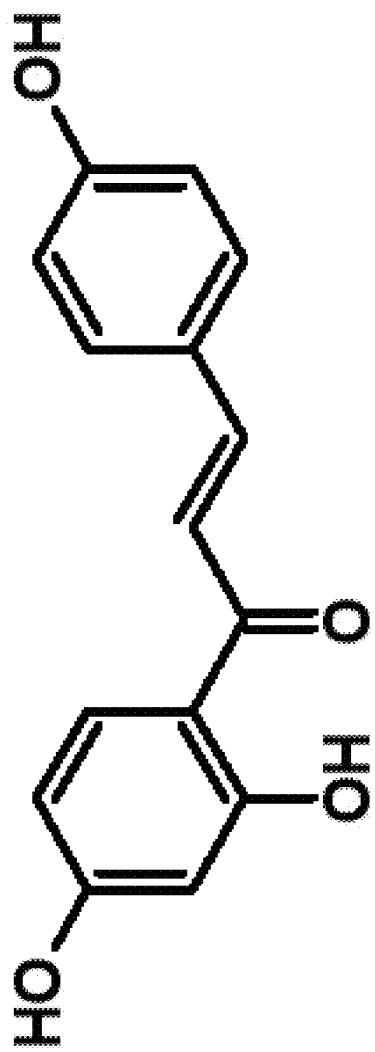
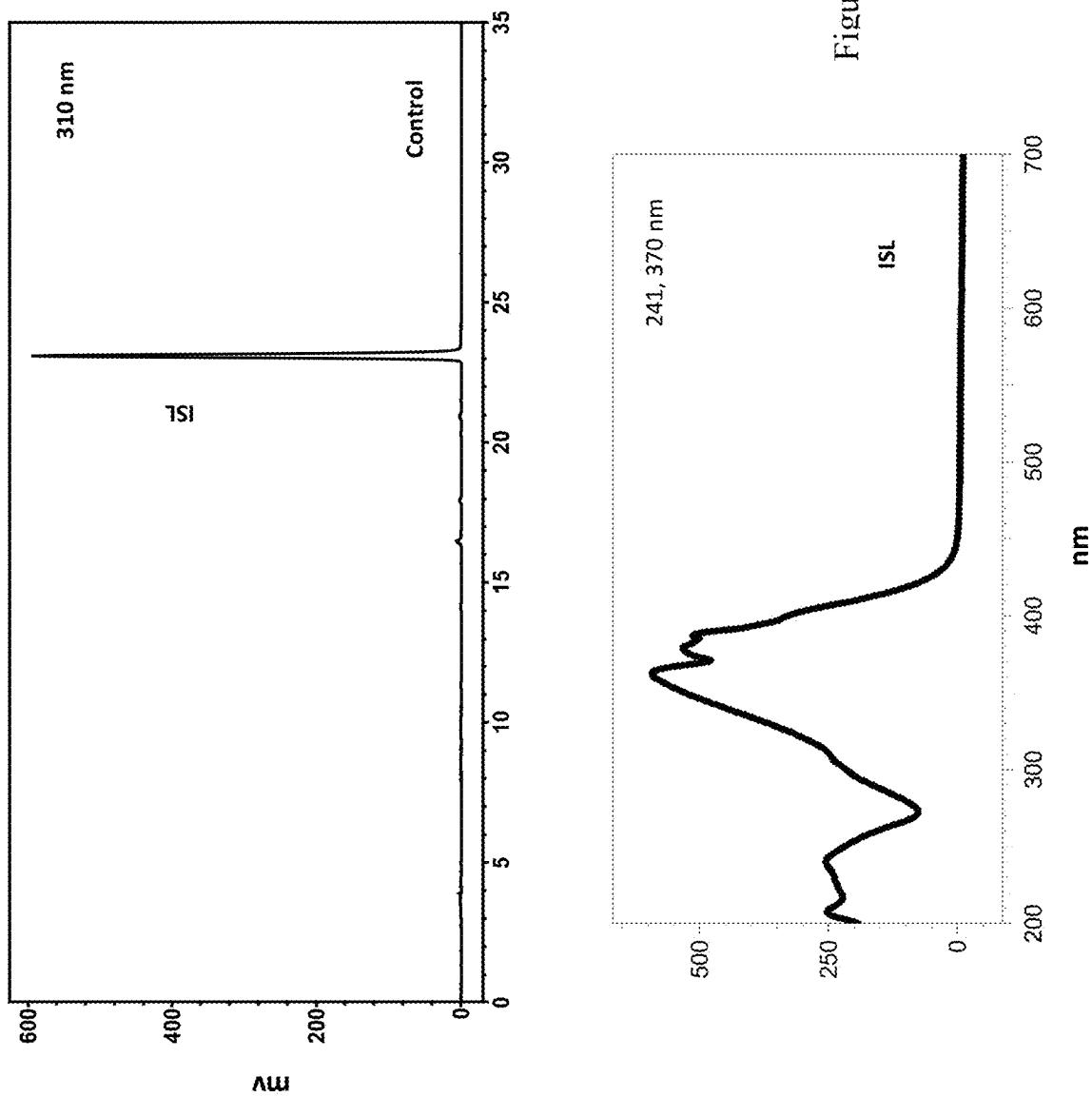


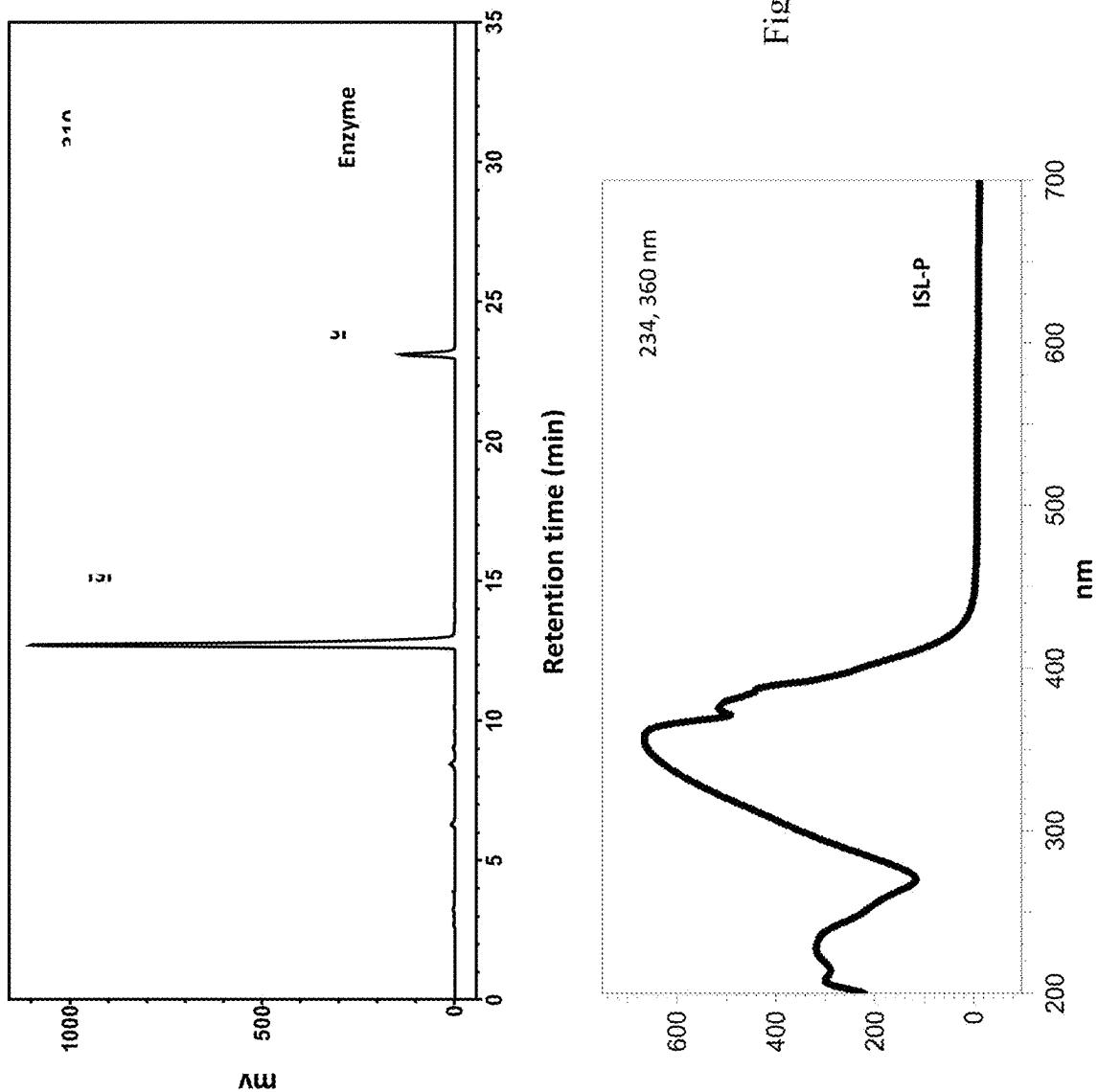
Figure 9(C)

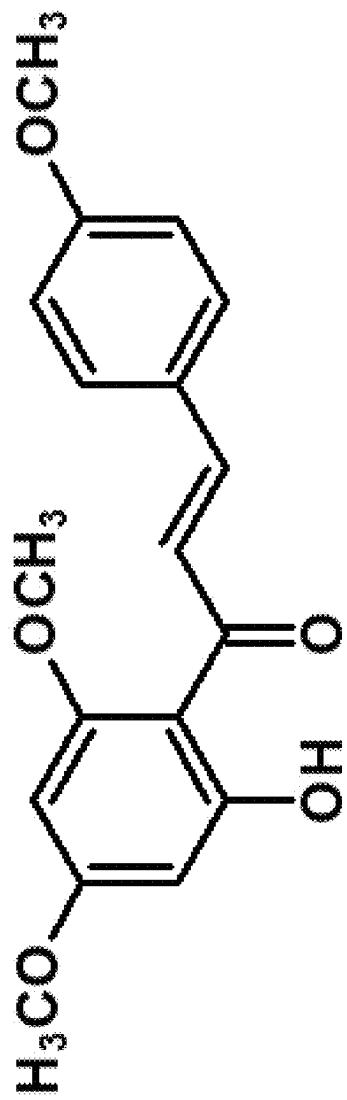


**isoliquiritigenin, ISL**  
*Glycyrrhiza glabra*

Figure.10(A)







**flavokawain A, FKA**  
*Piper methysticum*

Figure 11(A)

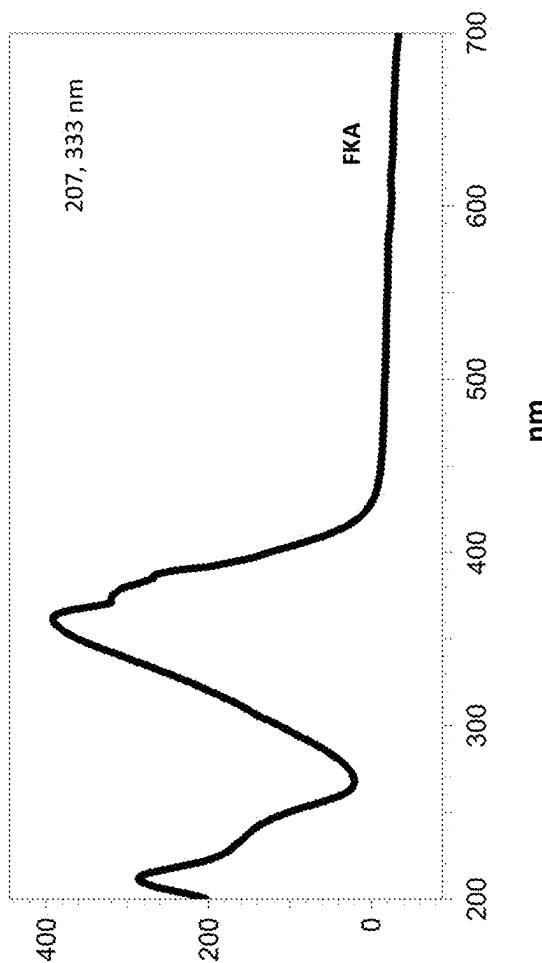
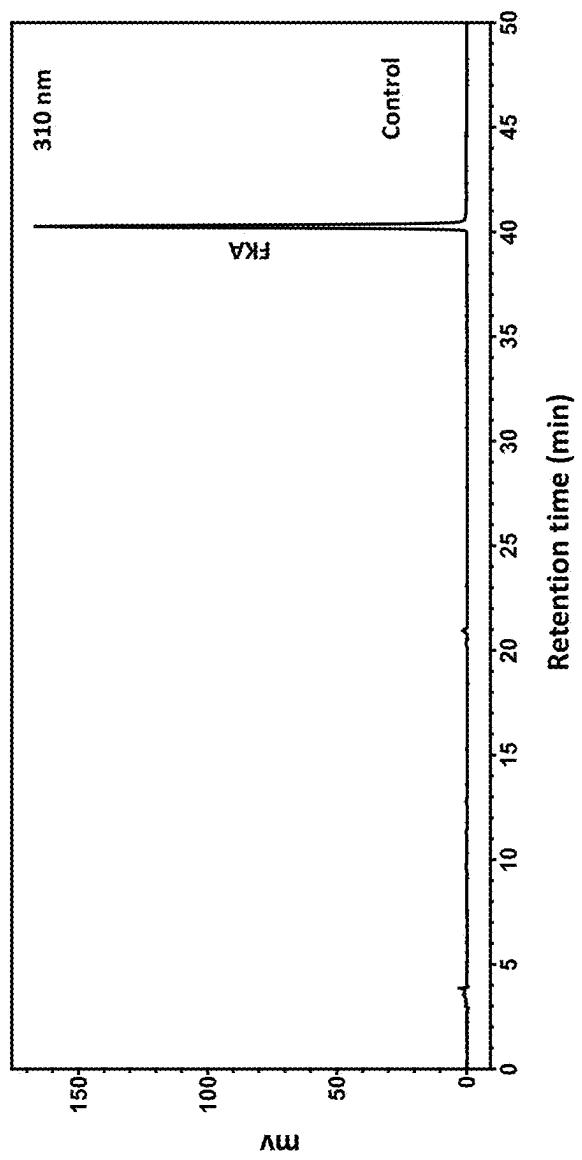


Figure 11(B)

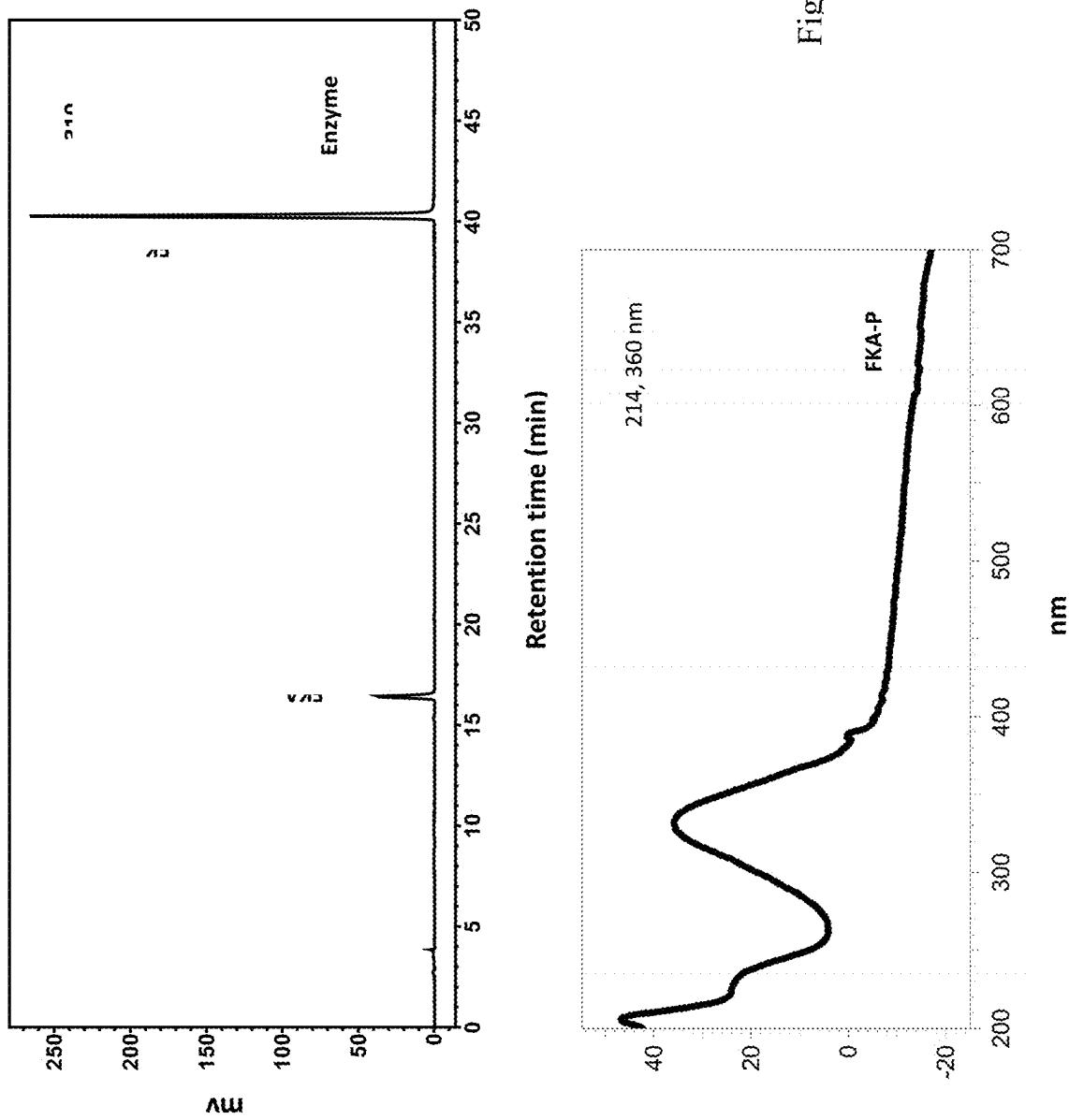


Figure.11(C)

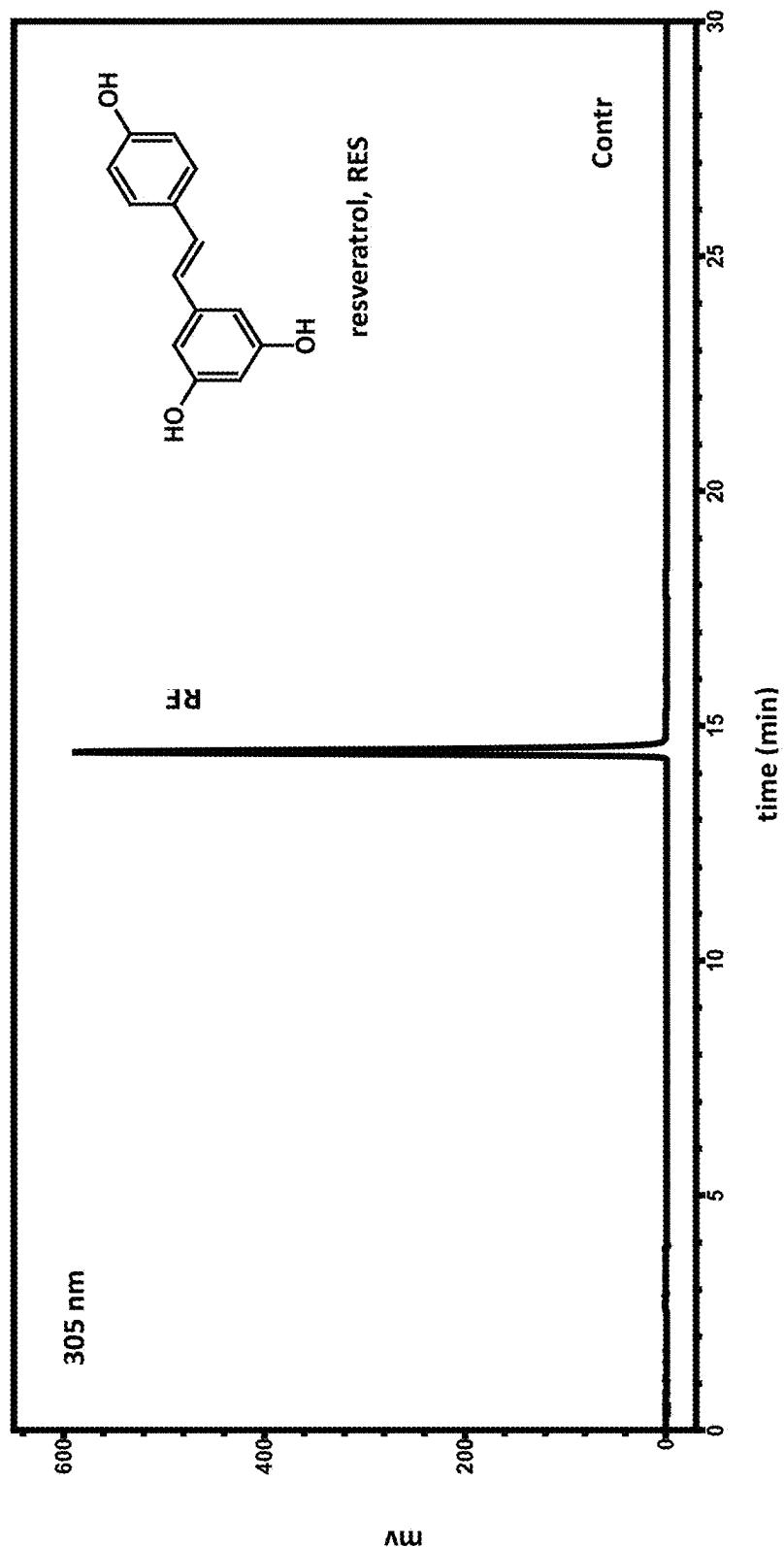


Figure 12(A)

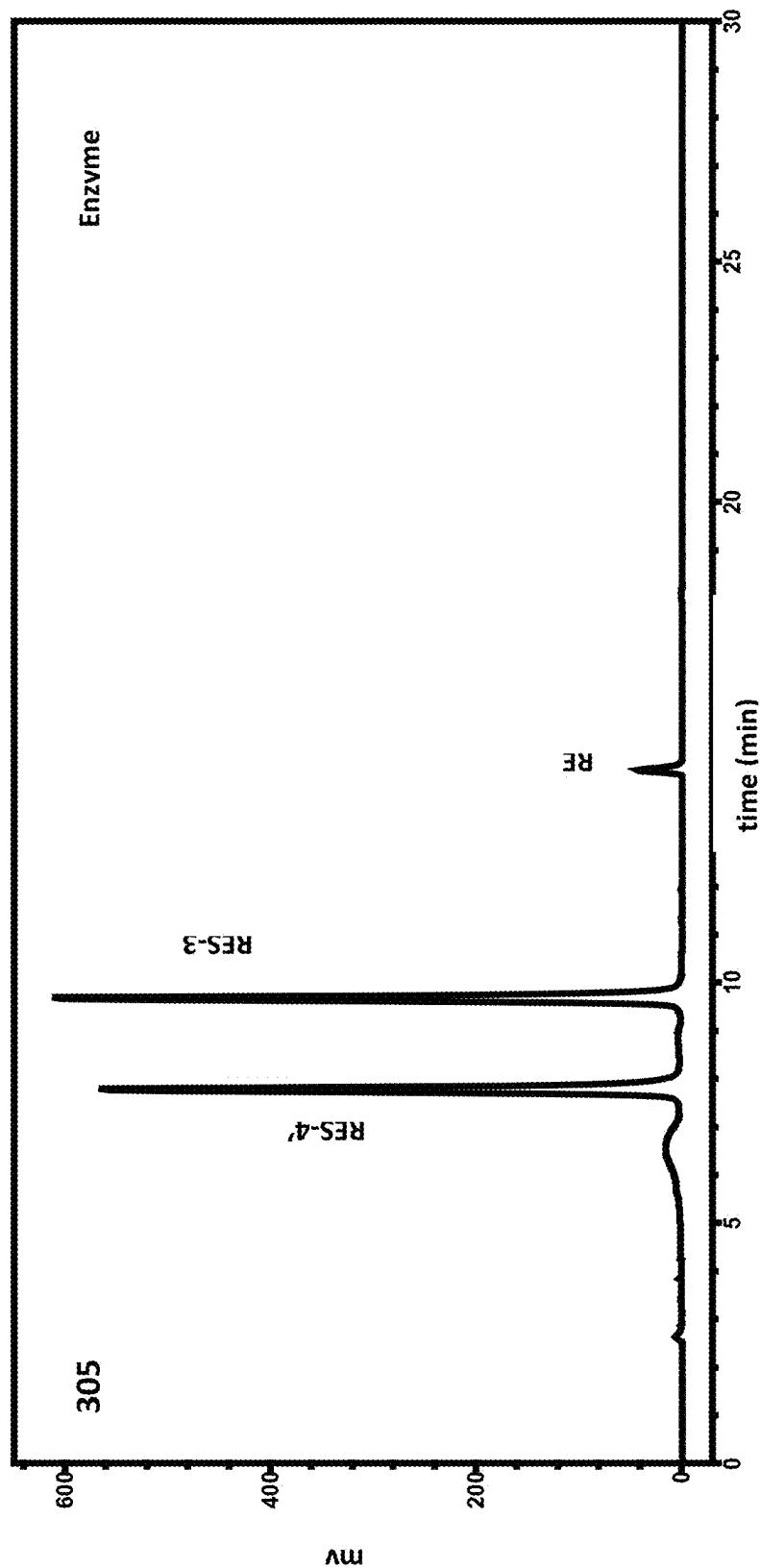


Figure 12(B)

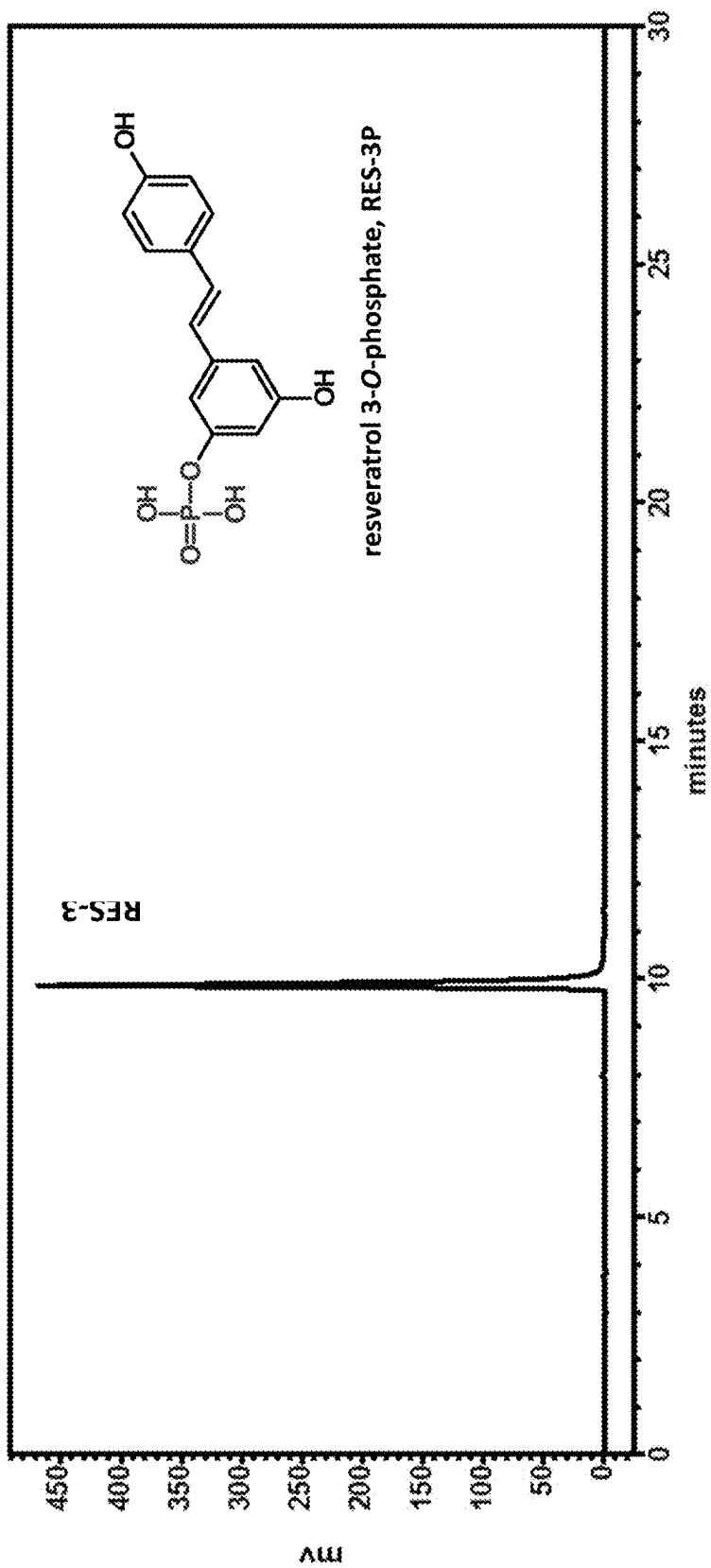


Figure 12(C)

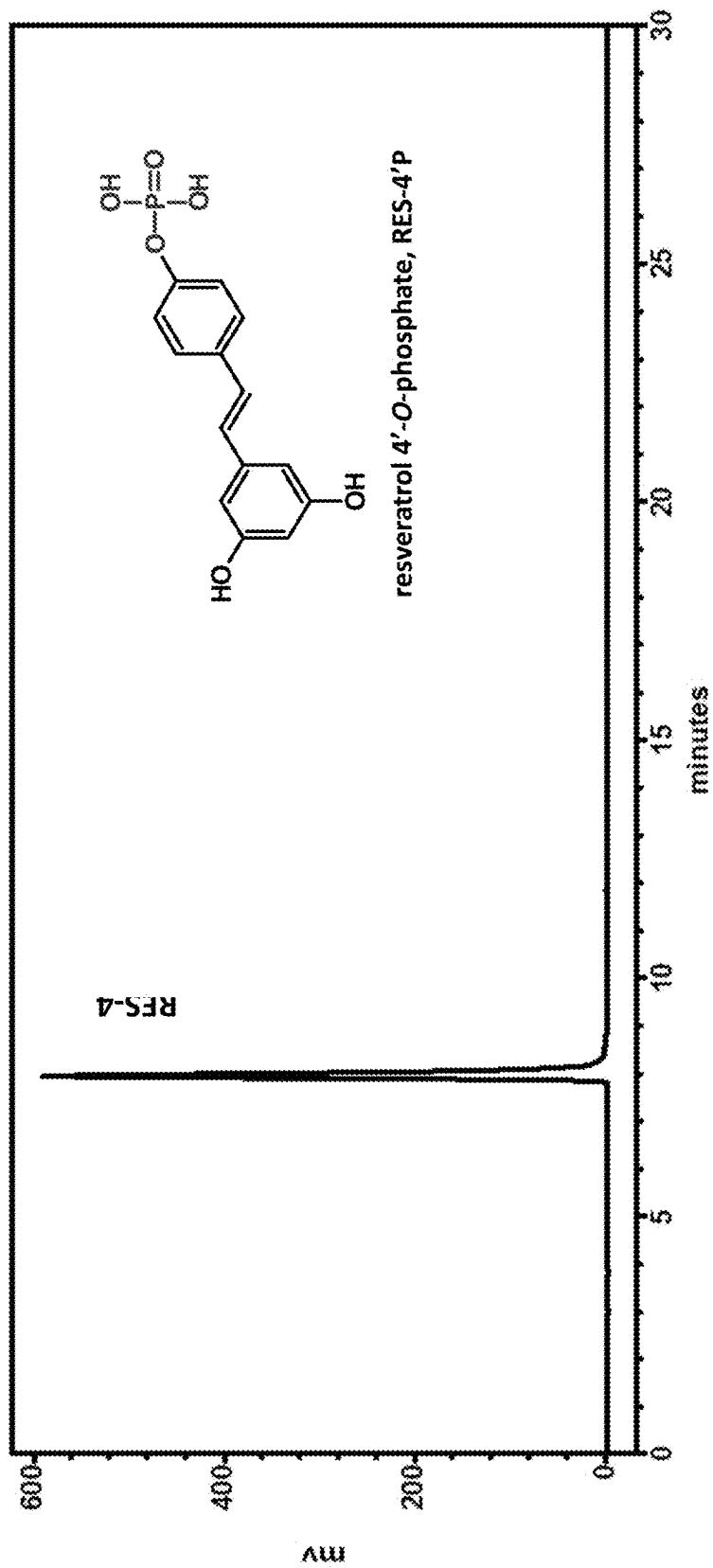


Figure.12(D)

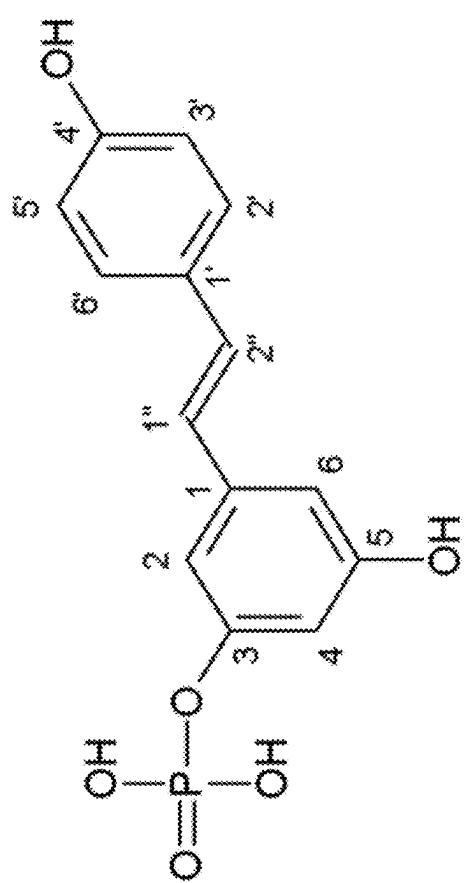


Figure.13(A)

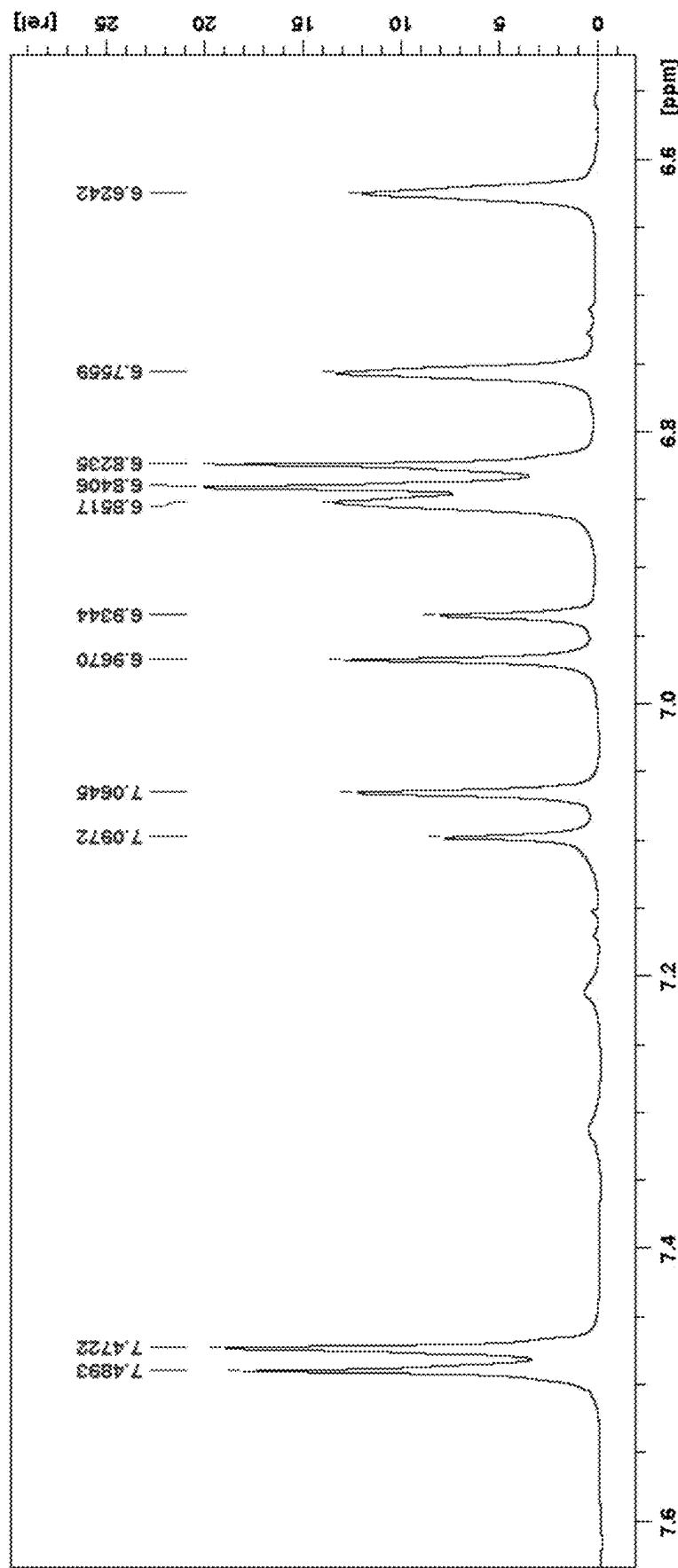


Figure 13(B)

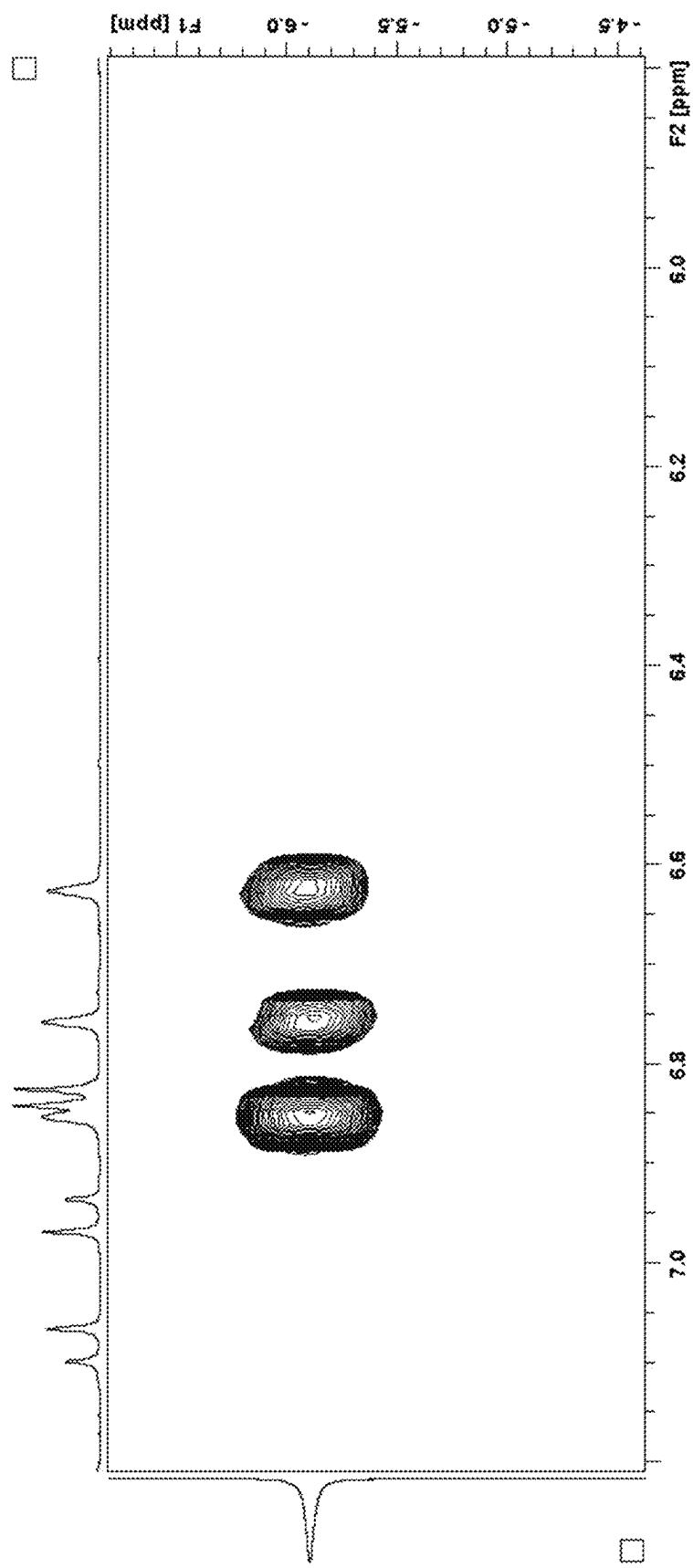


Figure.13(C)

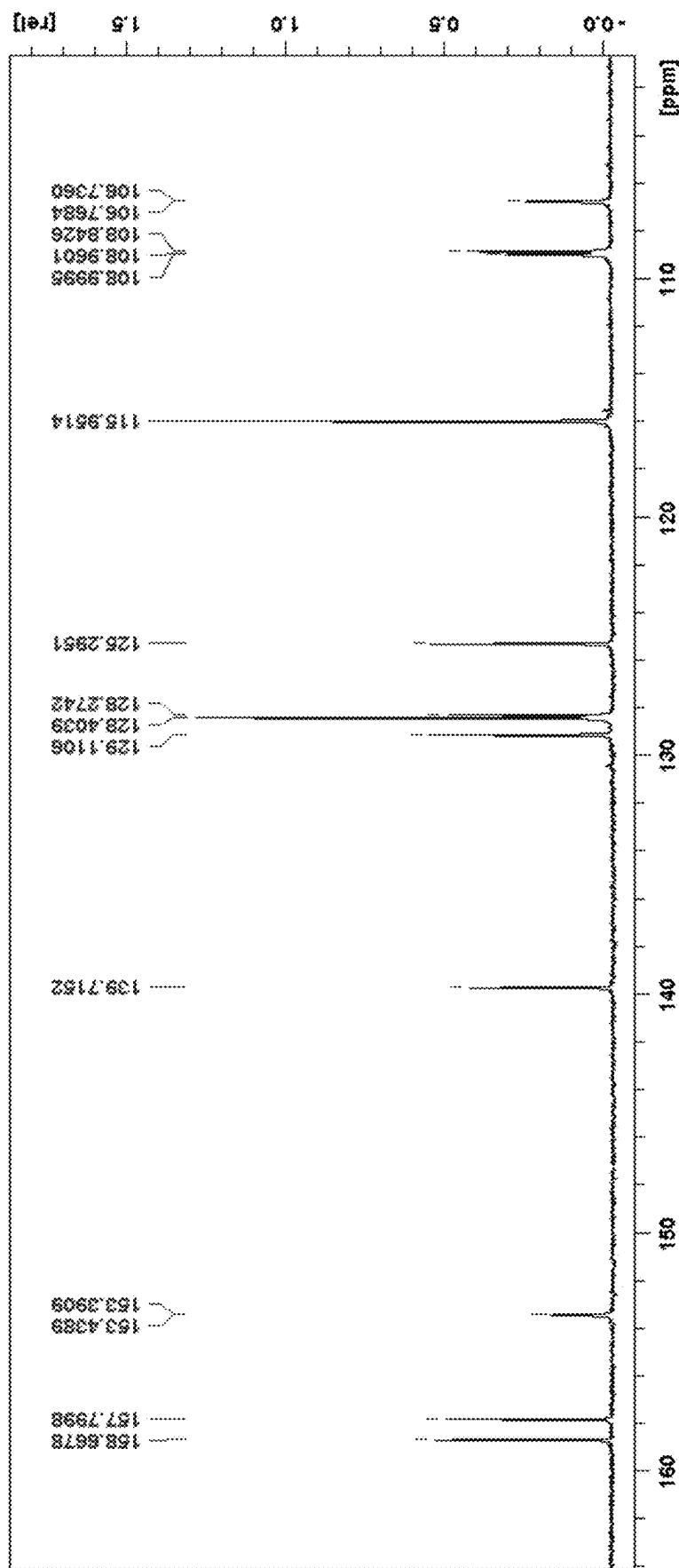


Figure 13(D)

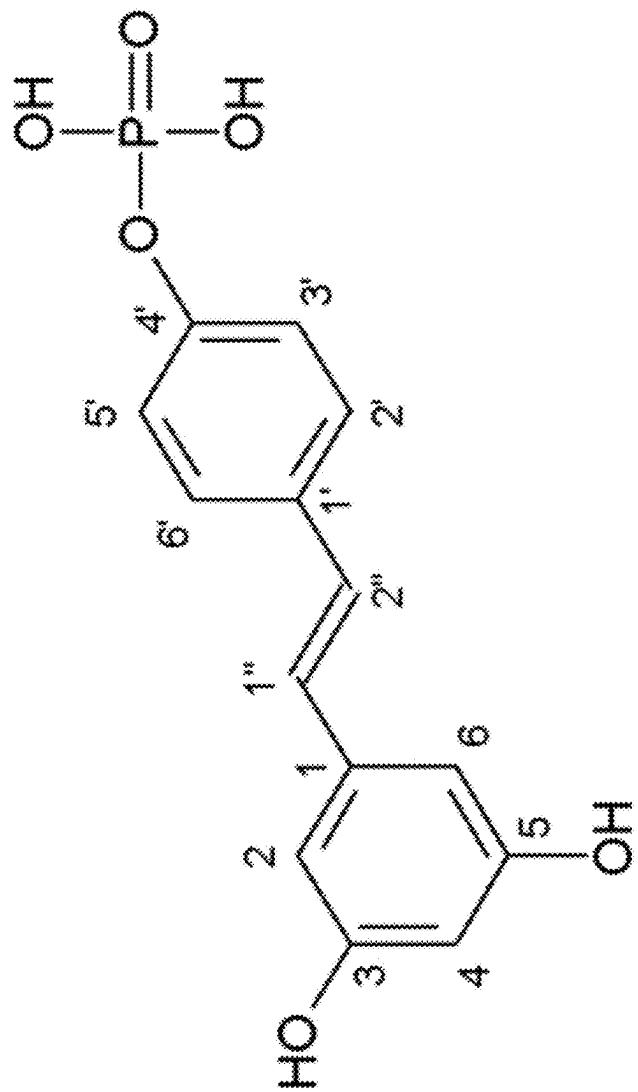


Figure.14(A)

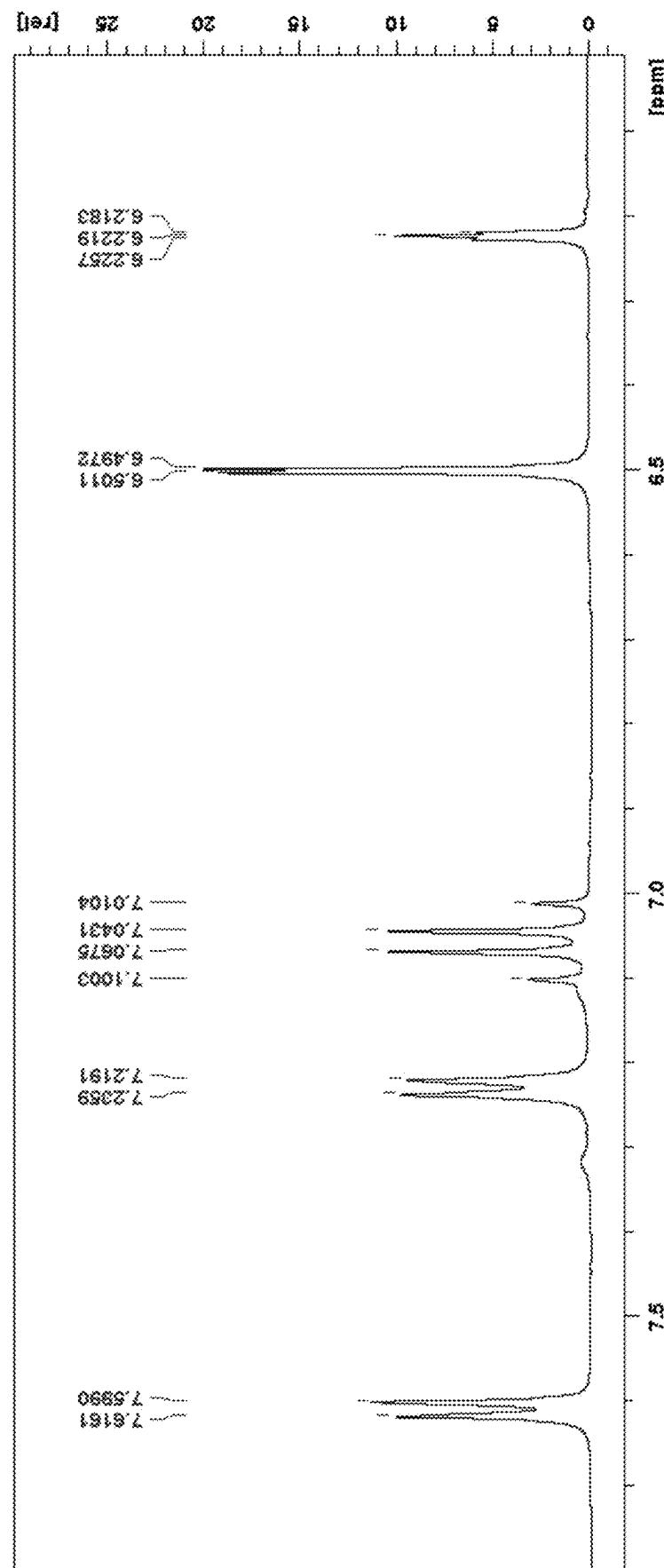


Figure.14(B)

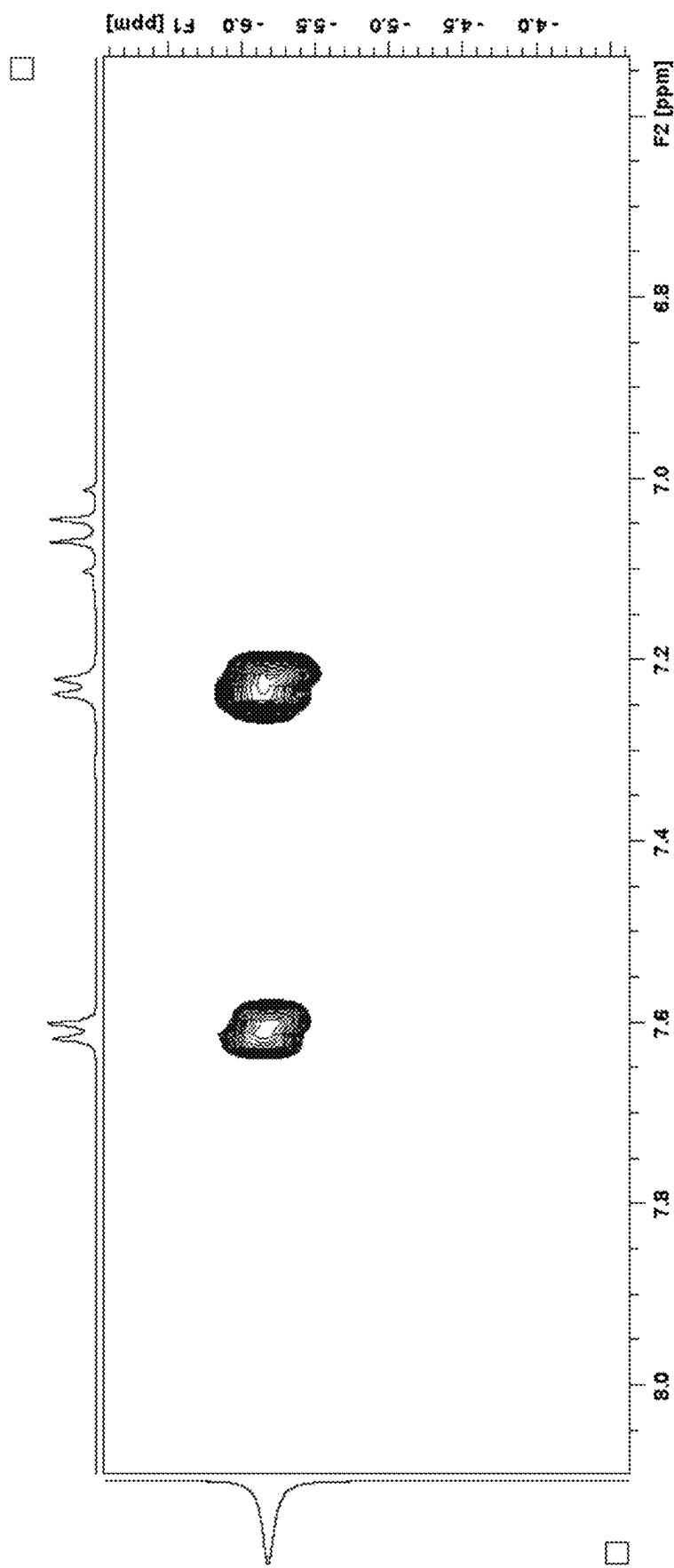


Figure 14(C)

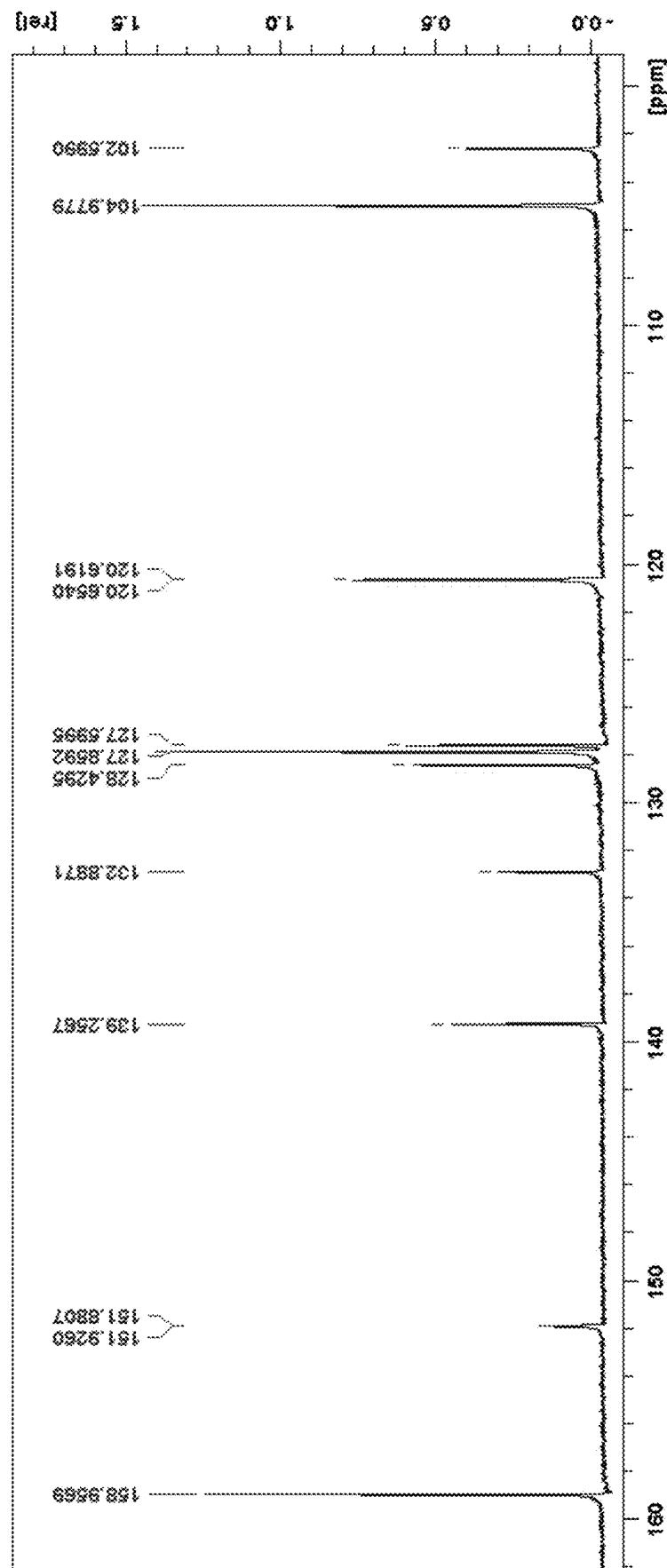
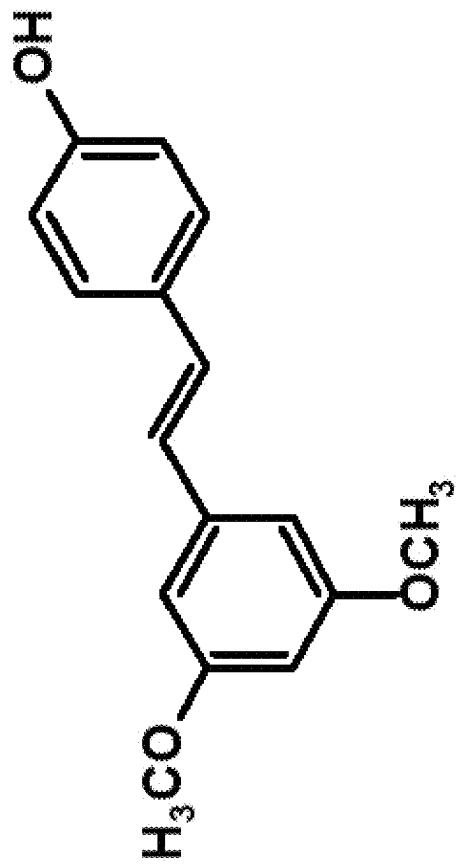


Figure.14(D)



## pterostilbene, PTER

Almonds, blueberries, grape leaves

Figure 15(A)

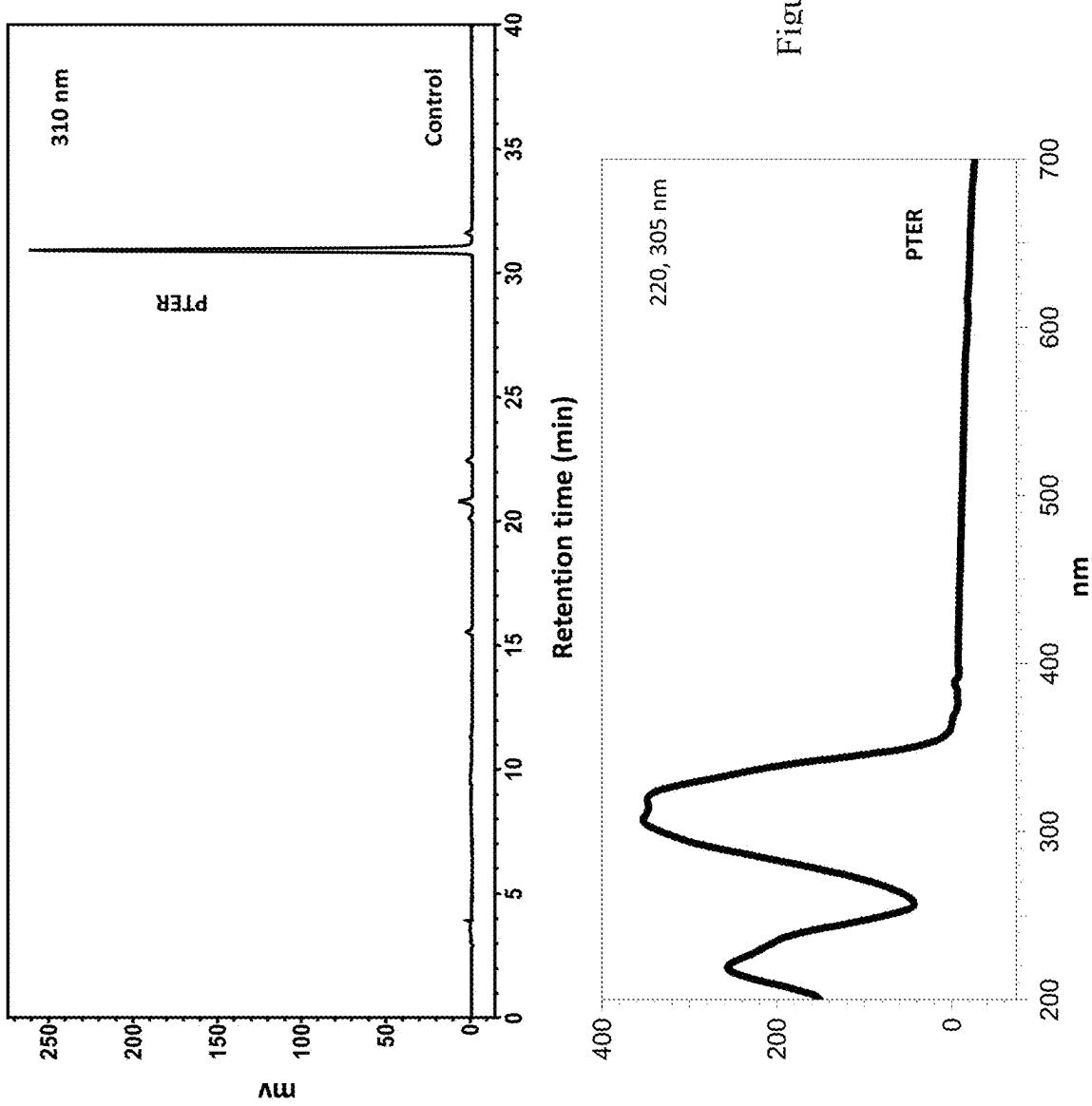


Figure 15(B)

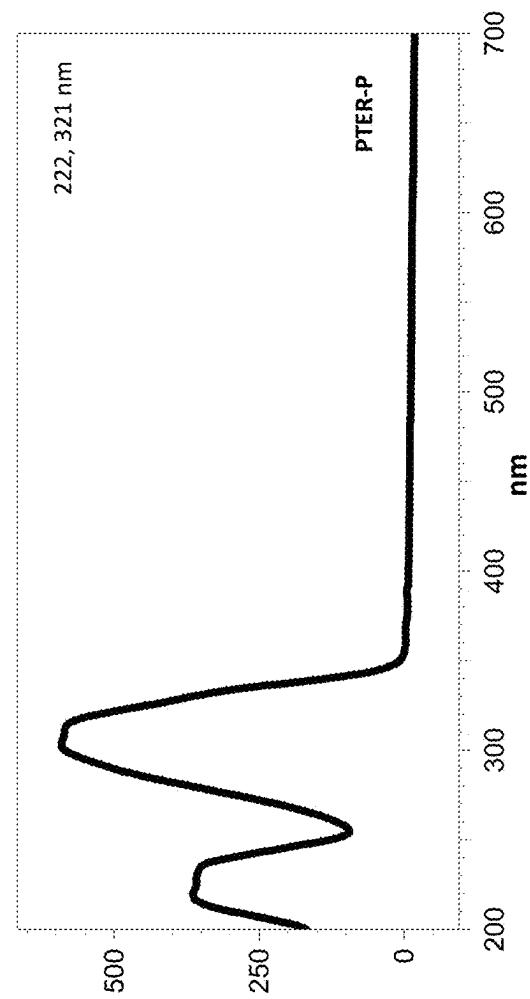
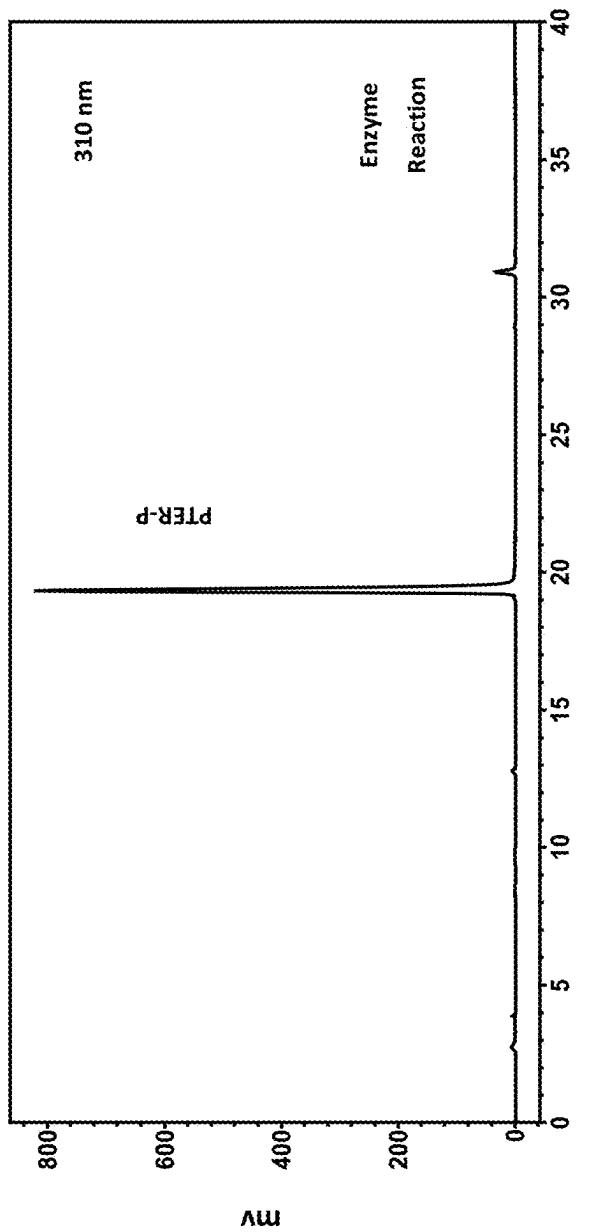
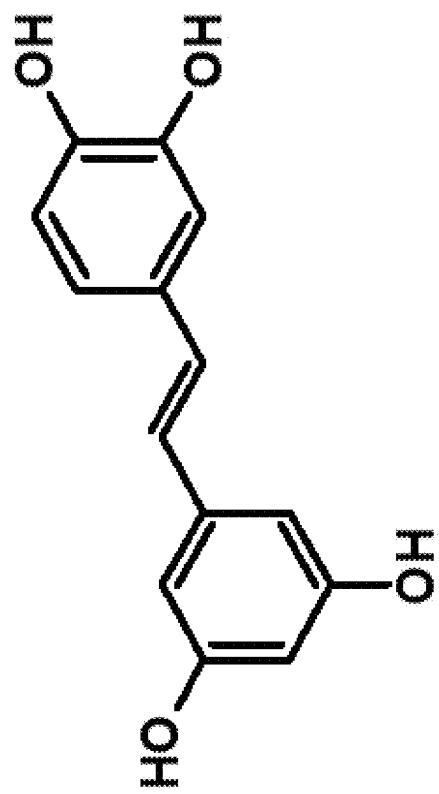


Figure.15(C)



**piceatannol, PCT**  
*Picea abies, Aiphanes horrida*

Figure.16(A)

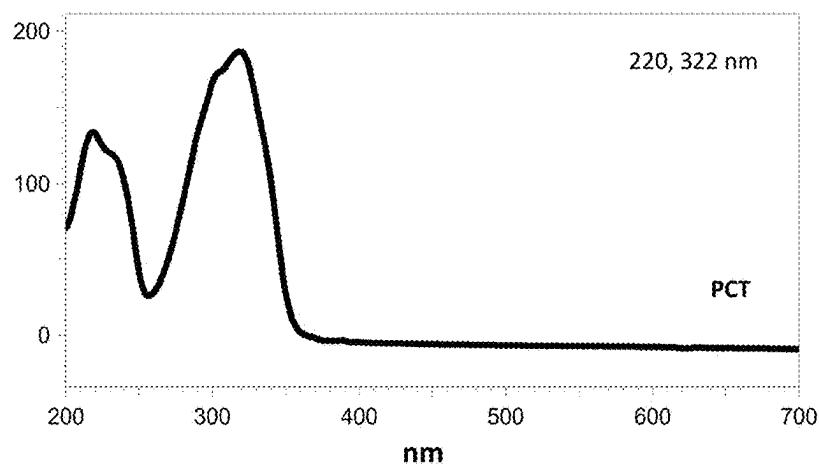
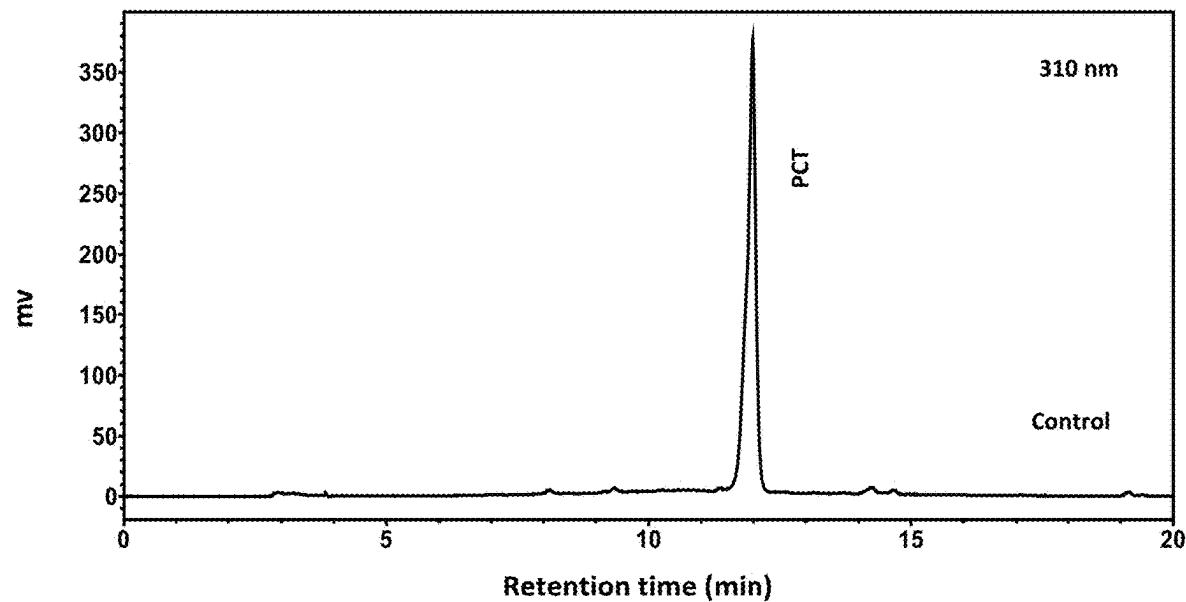
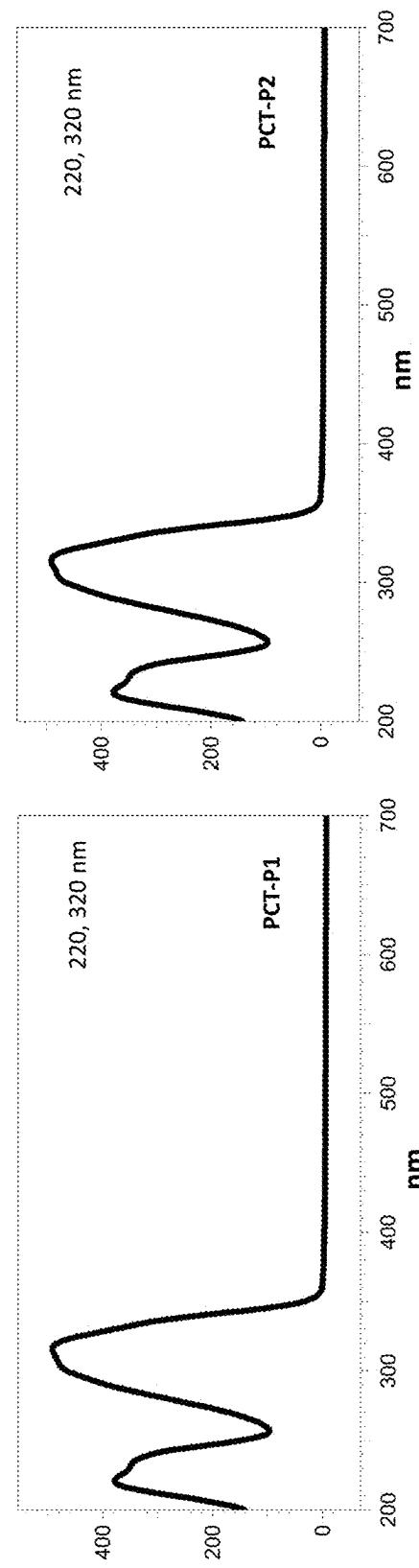
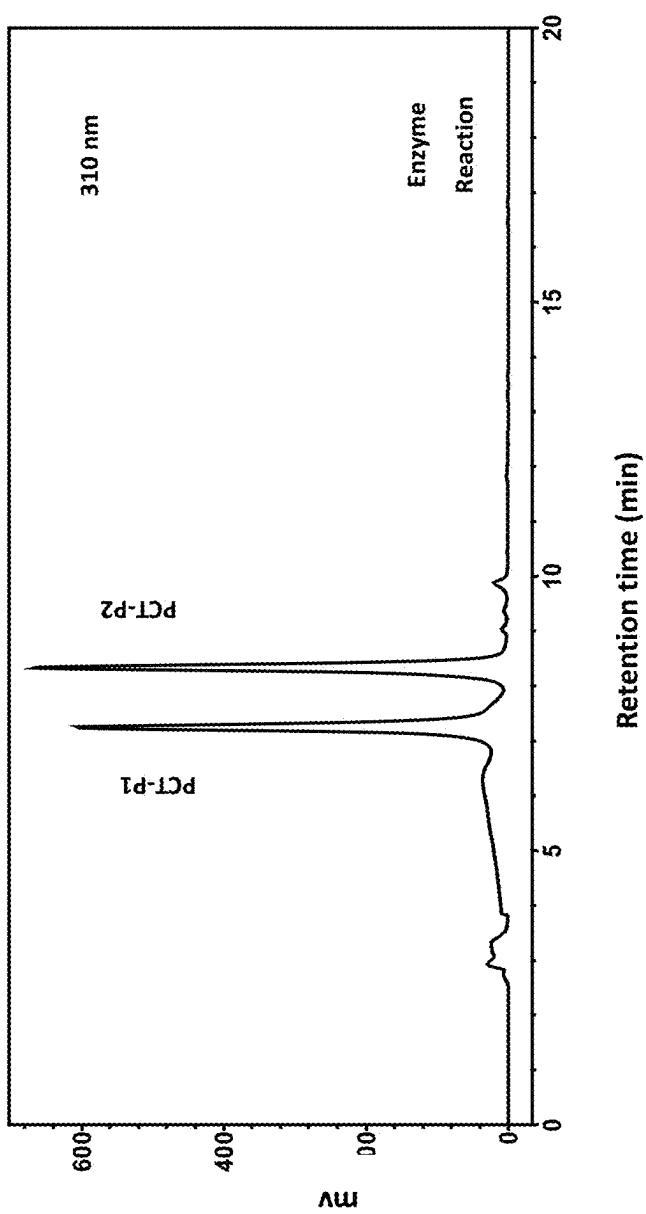
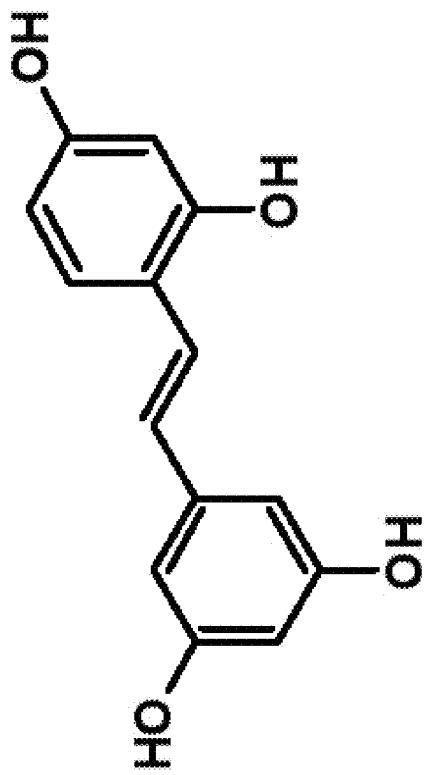


Figure.16(B)

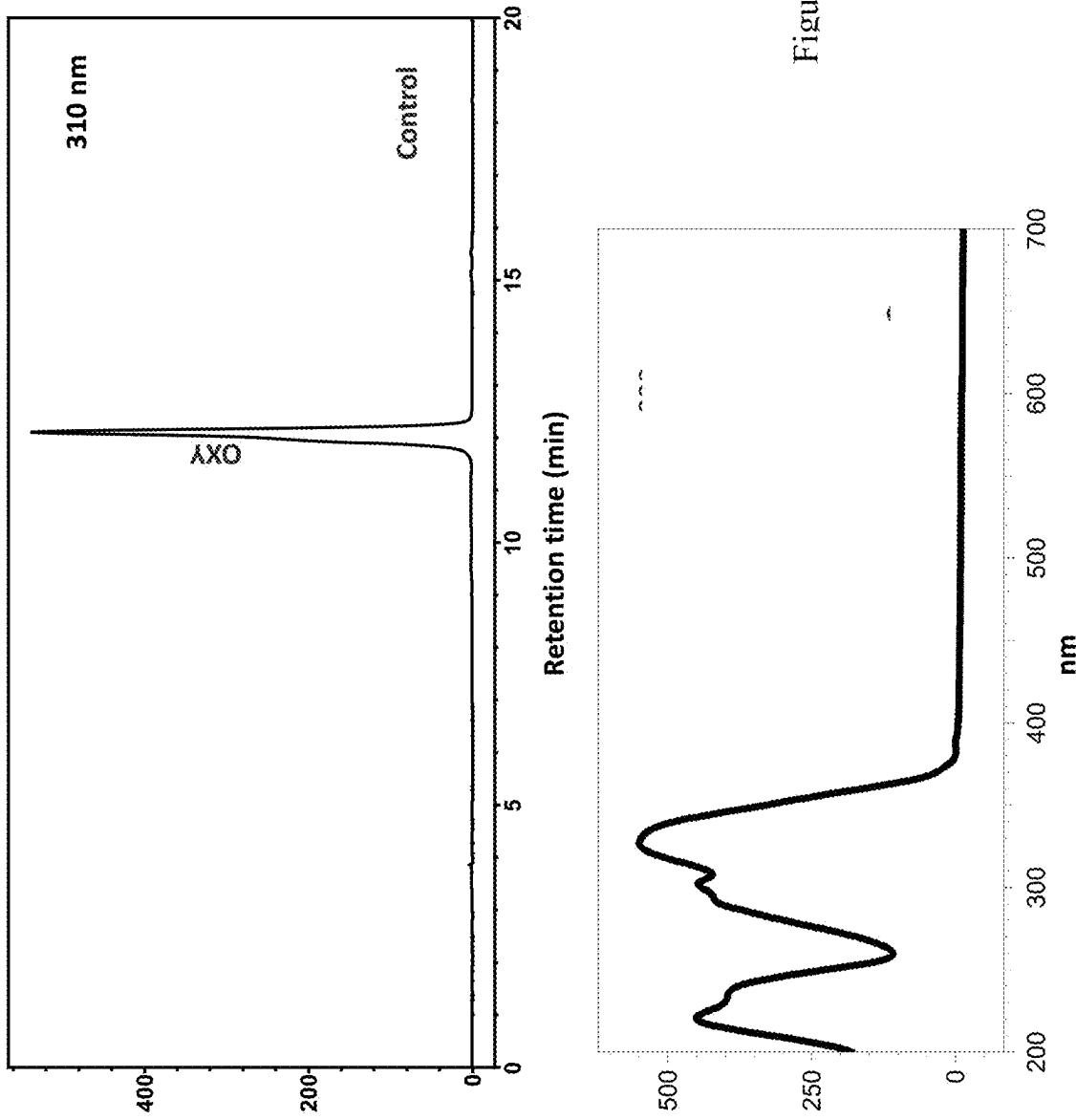
Figure.16(C)





**oxyresveratrol, OXY**  
*Artocarpus lacucha*

Figure.17(A)



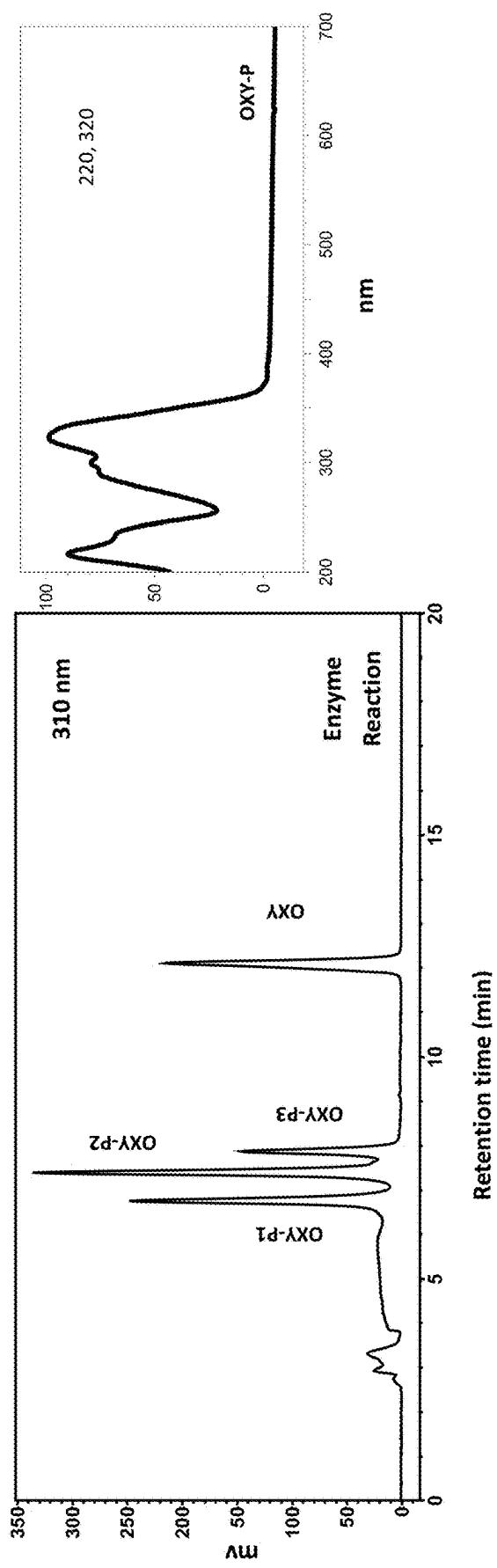
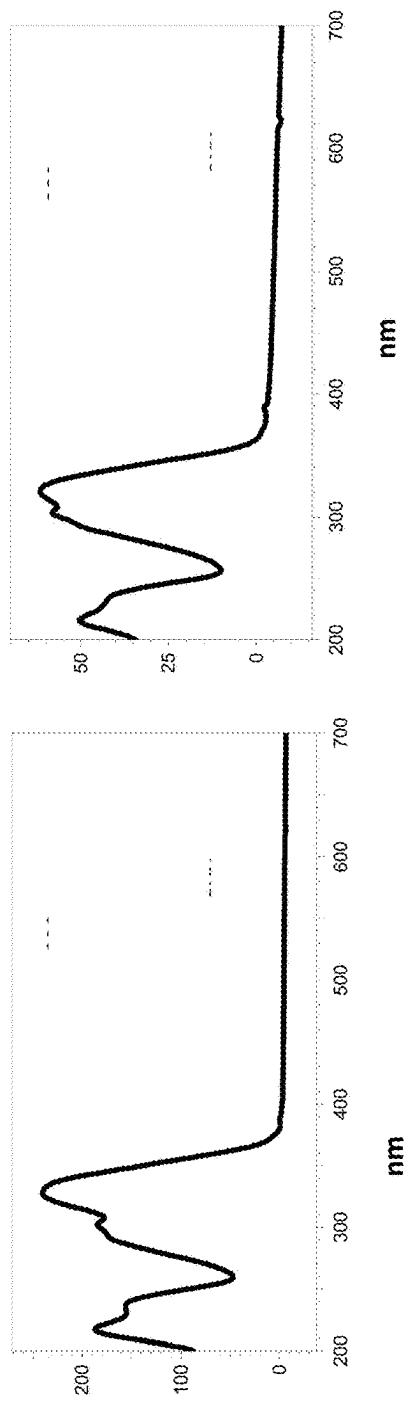
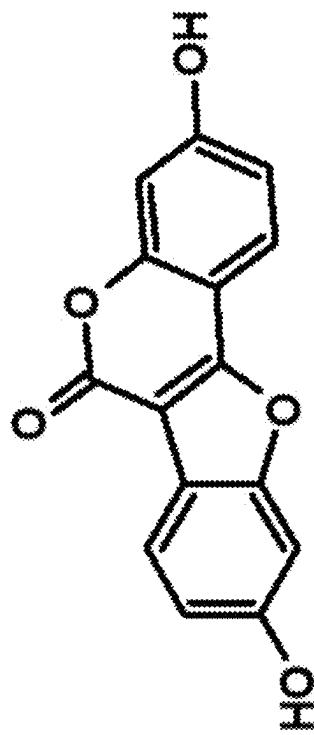


Figure.17(C)





**coumestrol, CUM**

*Medicago sativa*

Figure.18(A)

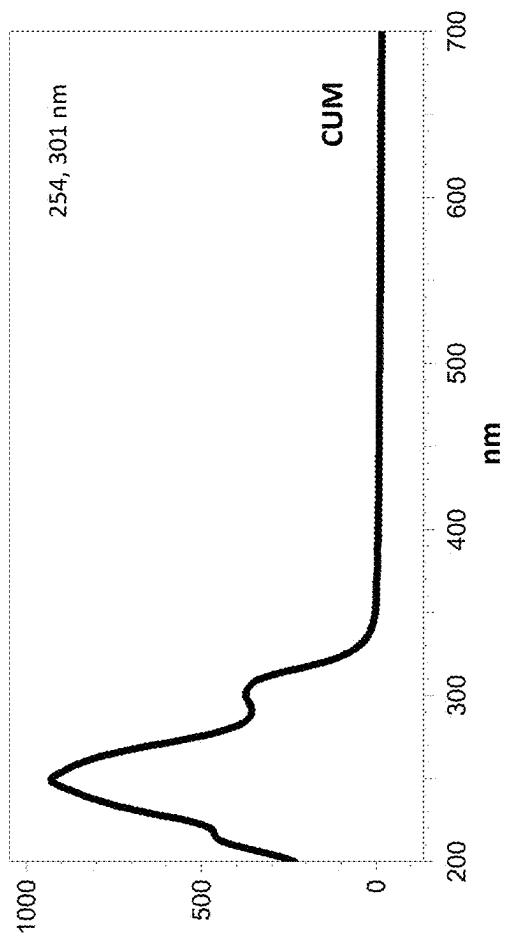
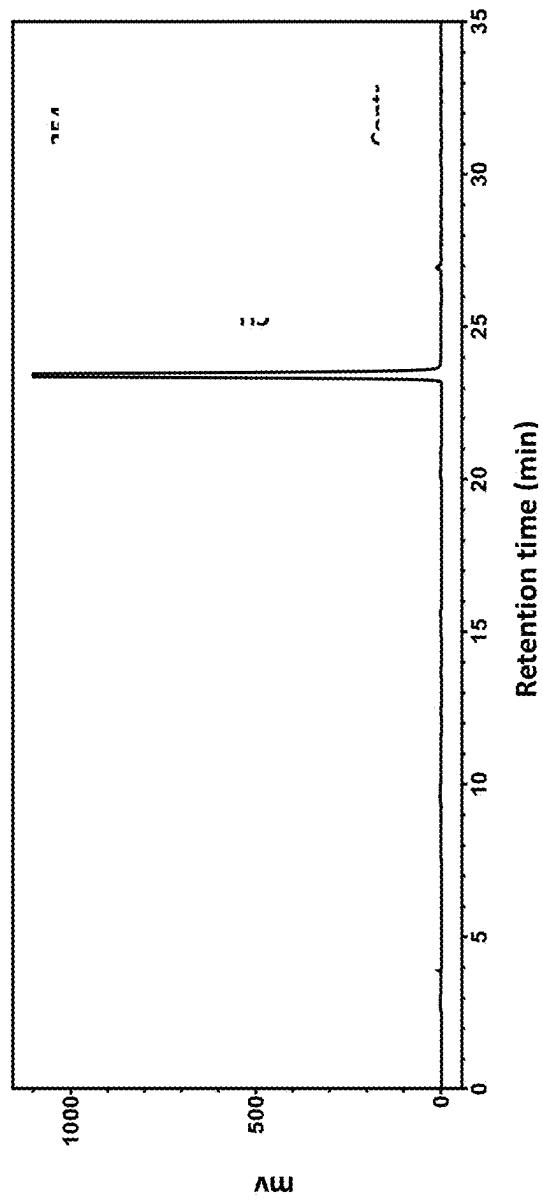


Figure.18(B)

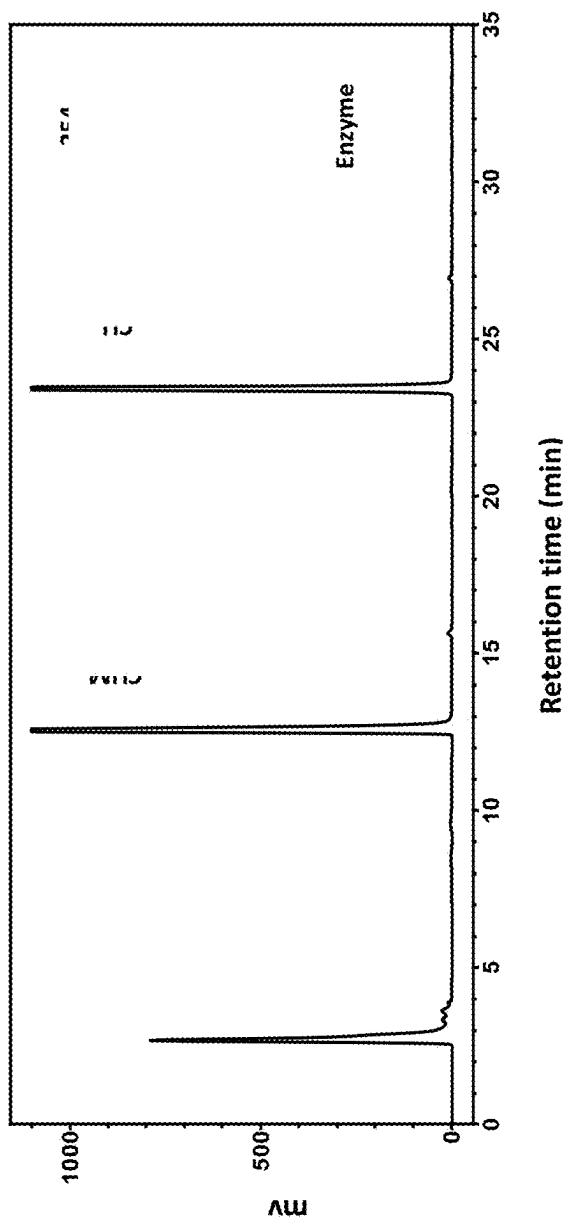
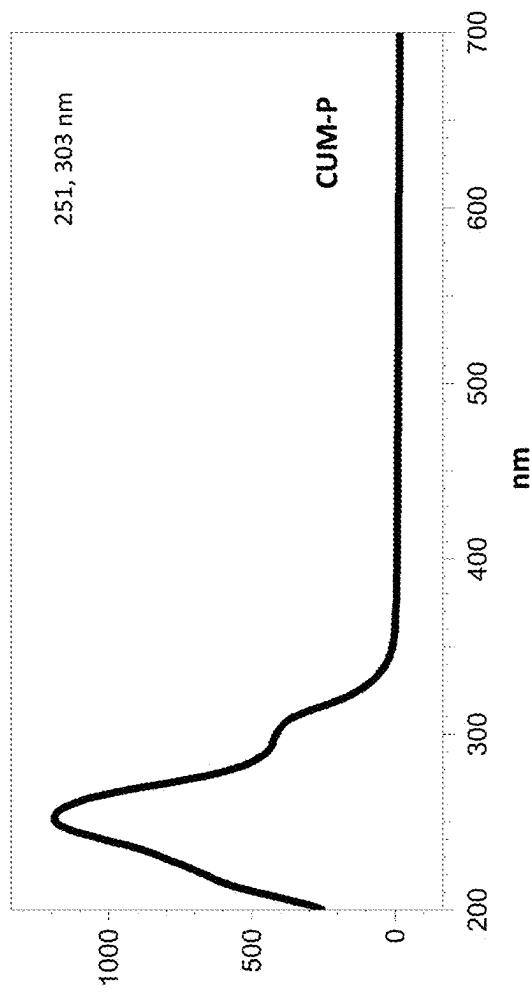


Figure.18(C)



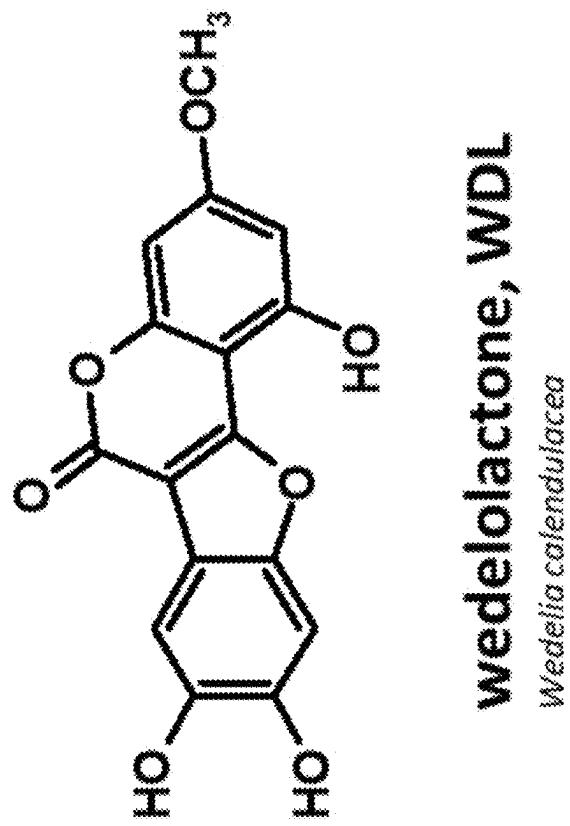
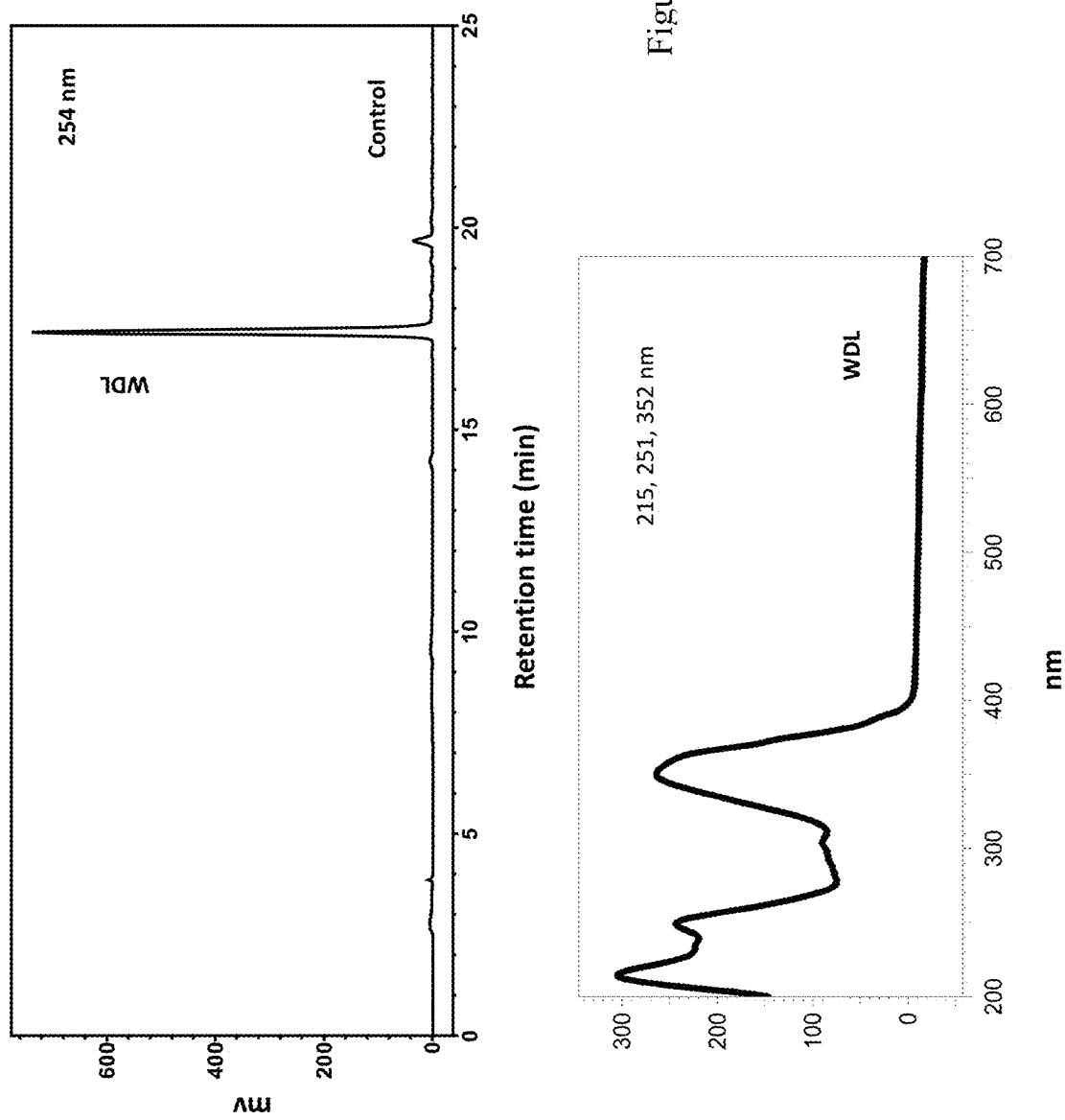
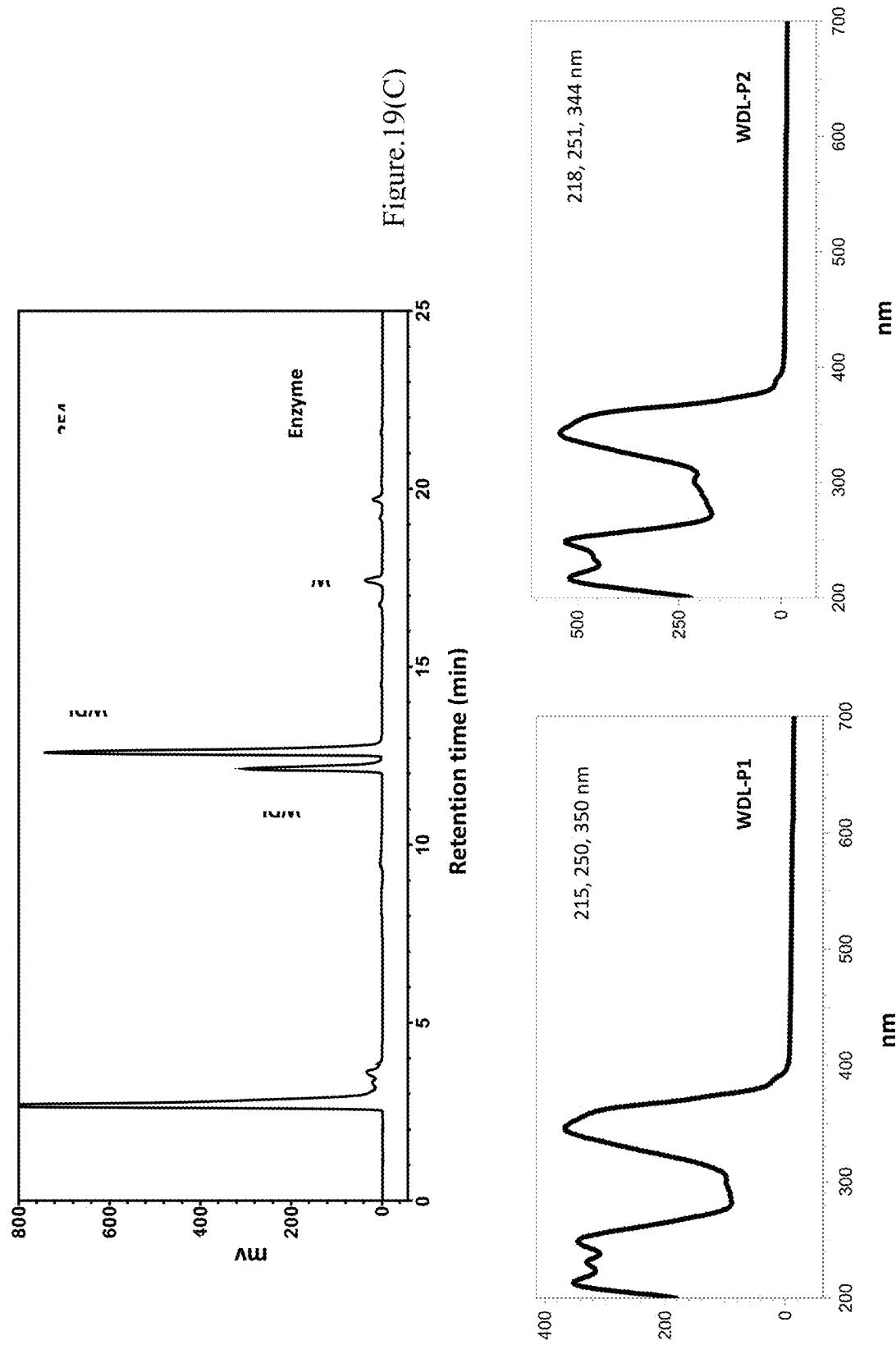
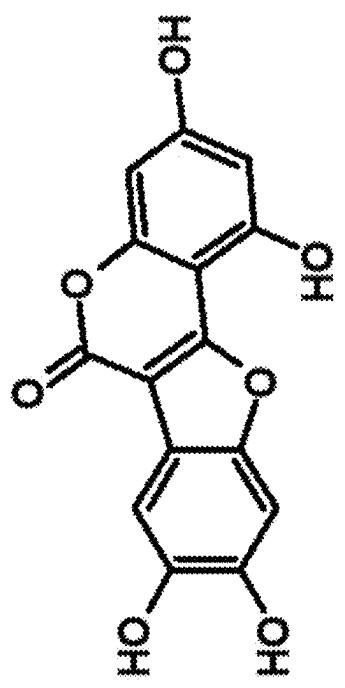


Figure.19(A)







**demethylwedelolactone, DimWDL**  
*Wedelia calendulacea*

Figure 20(A)

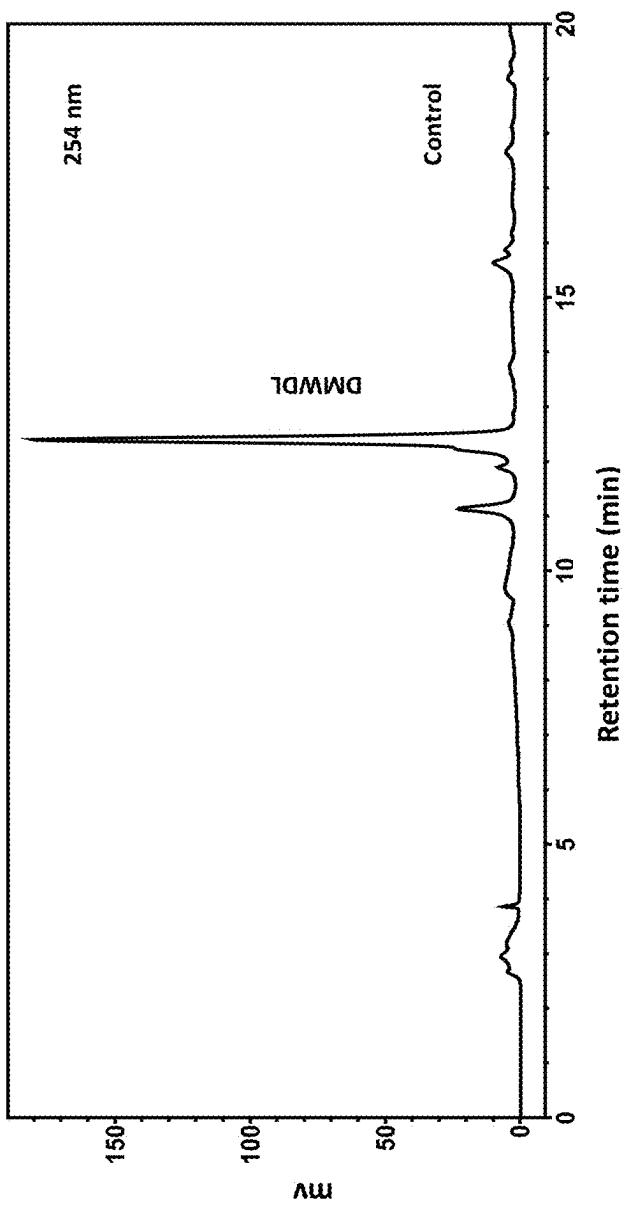
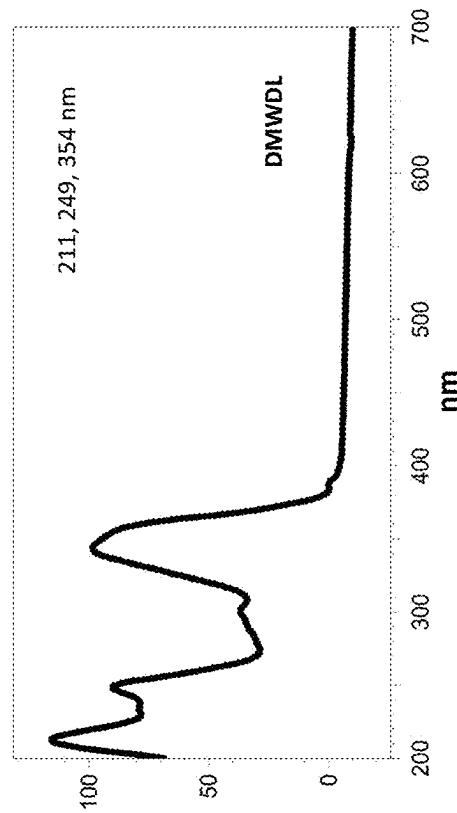
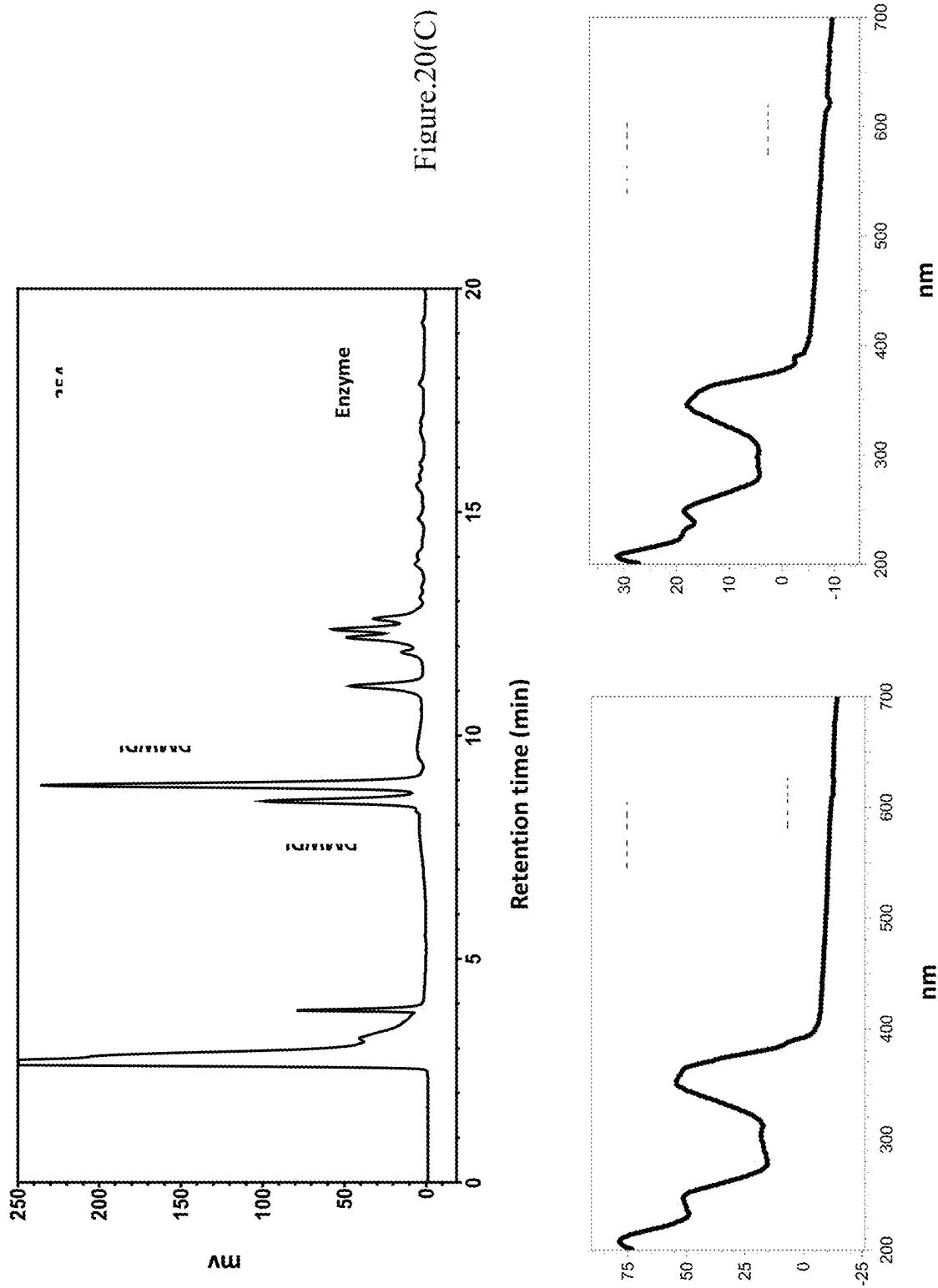
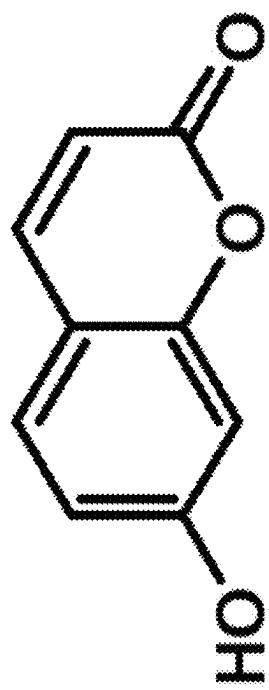


Figure.20(B)



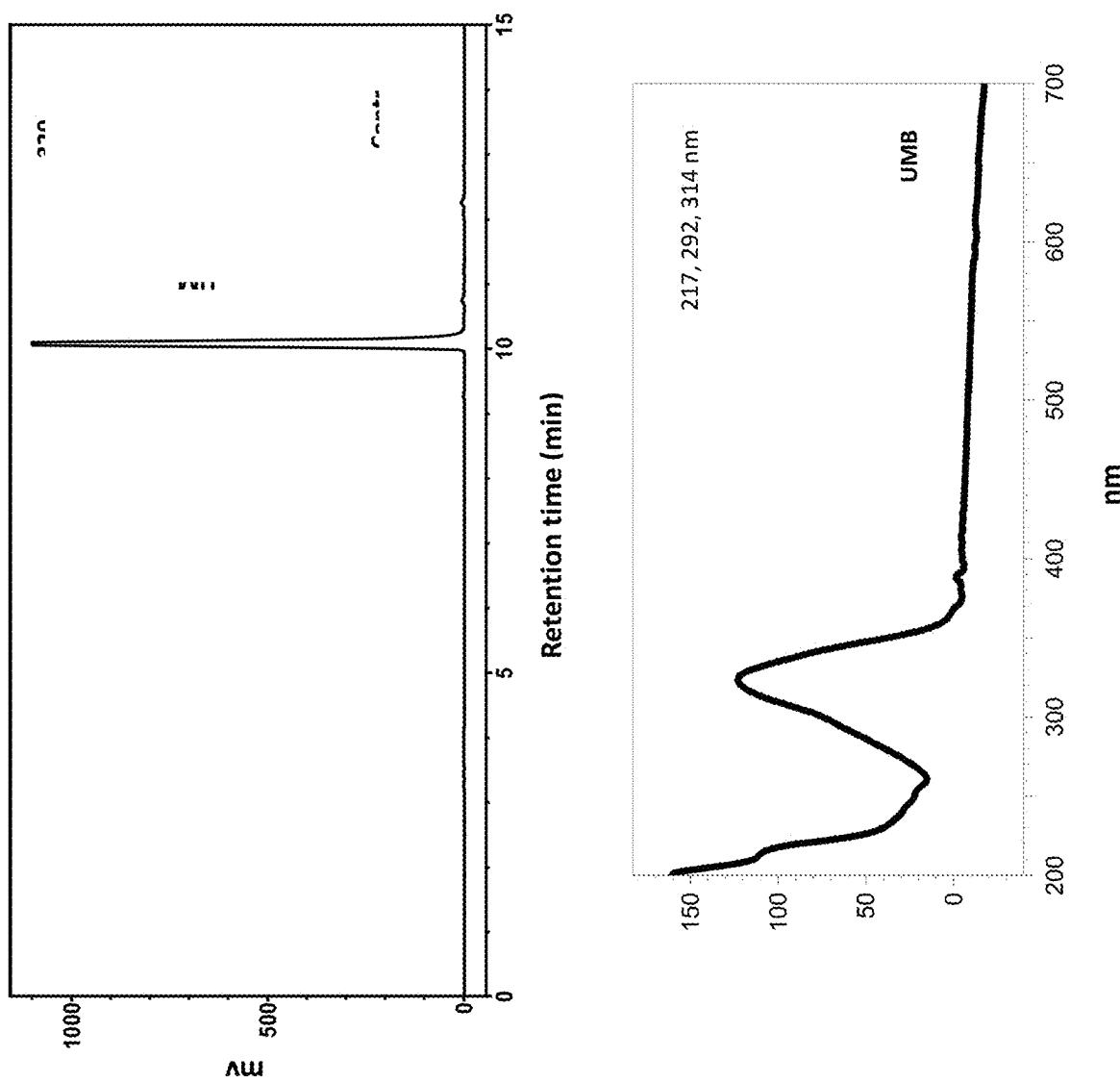




**umbelliferone, UMB**  
Synthetic compound

Figure.21(A)

Figure.21(B)



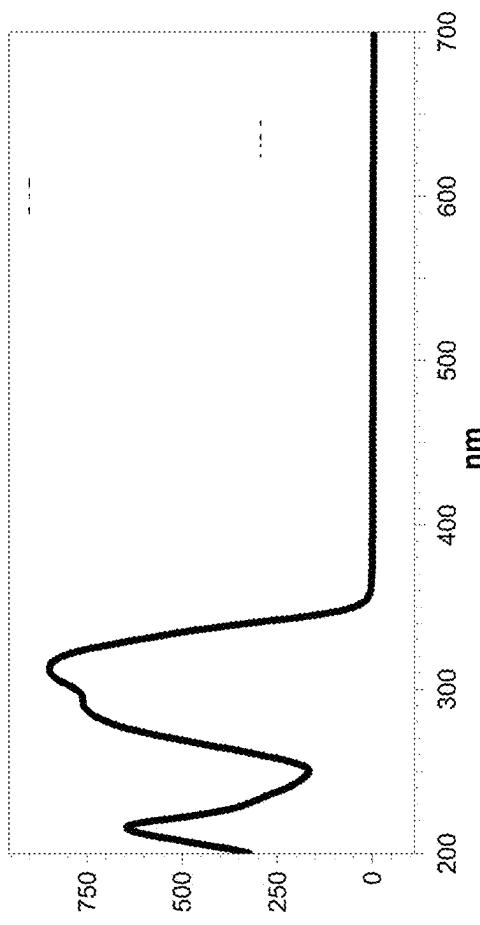
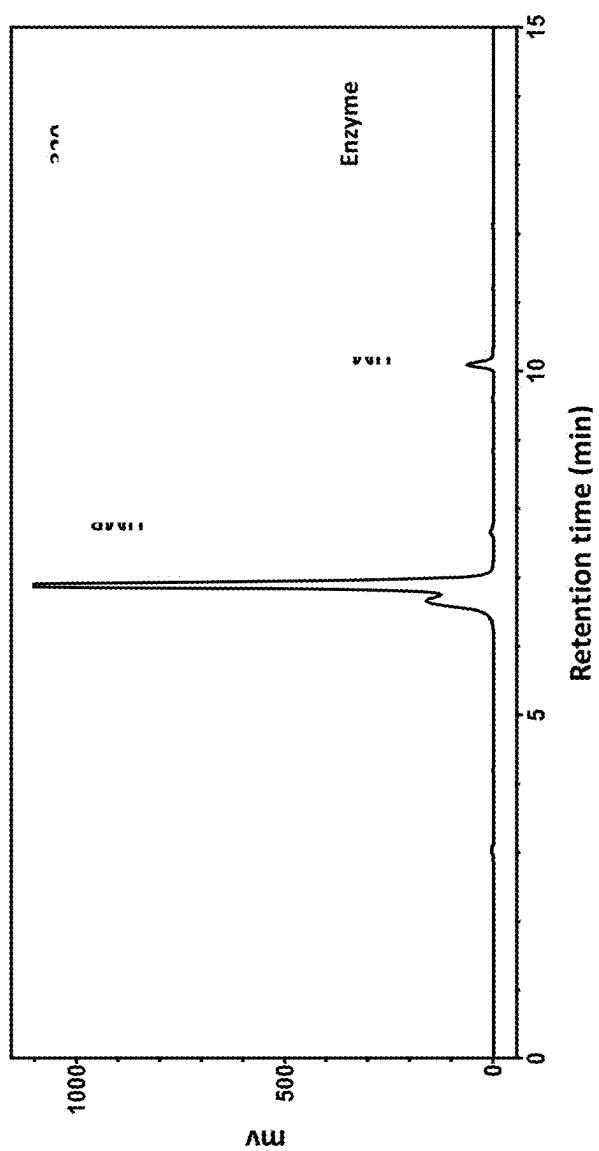


Figure 21(C)

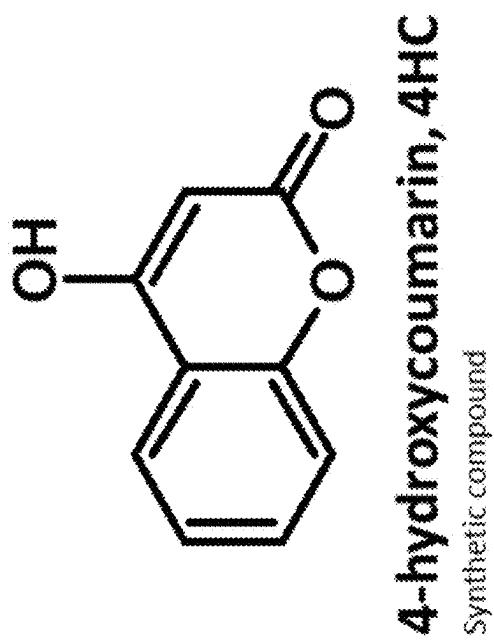


Figure.22(A)

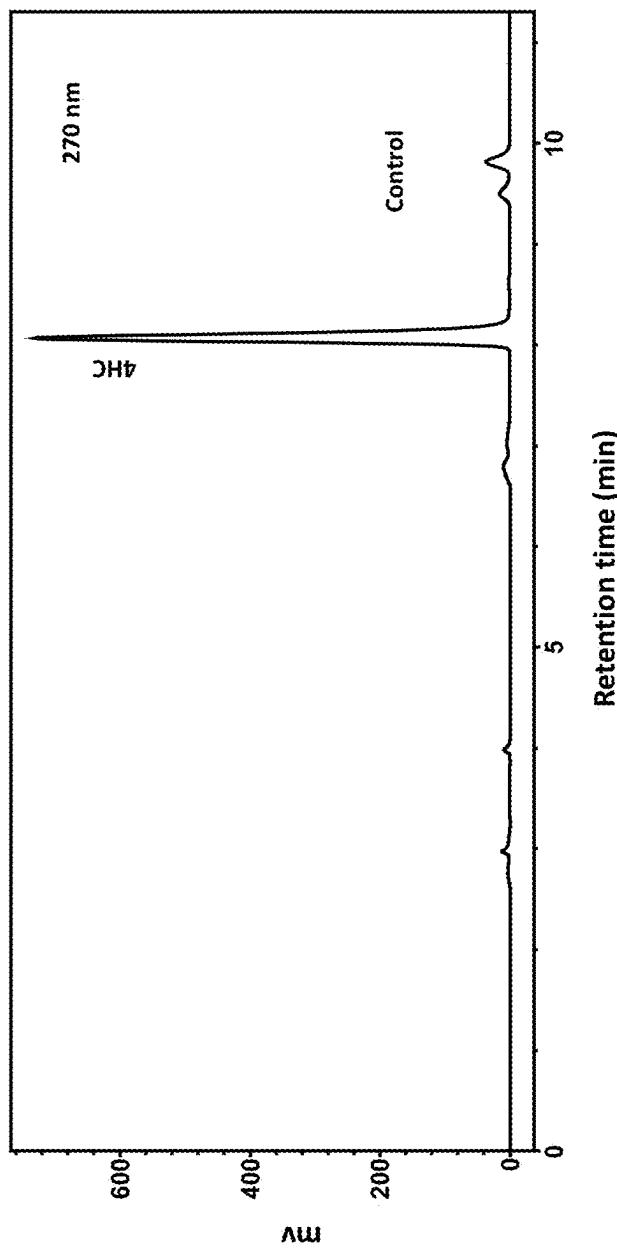
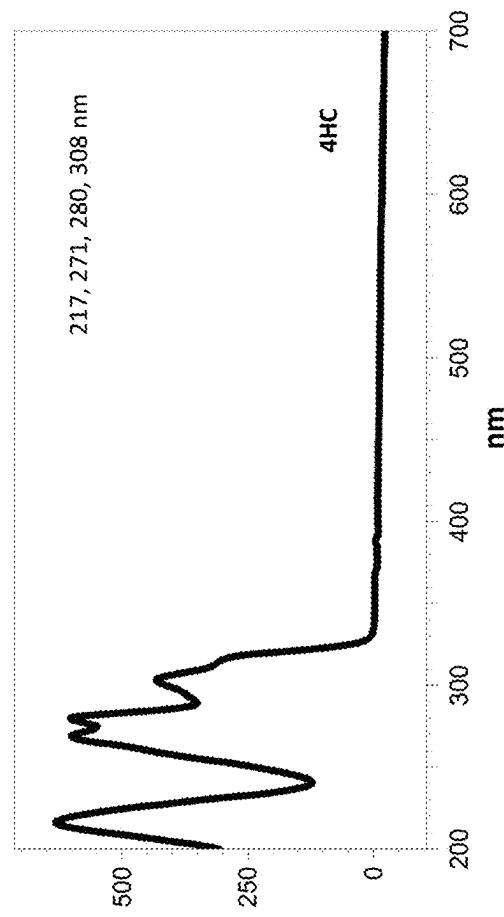


Figure.22(B)



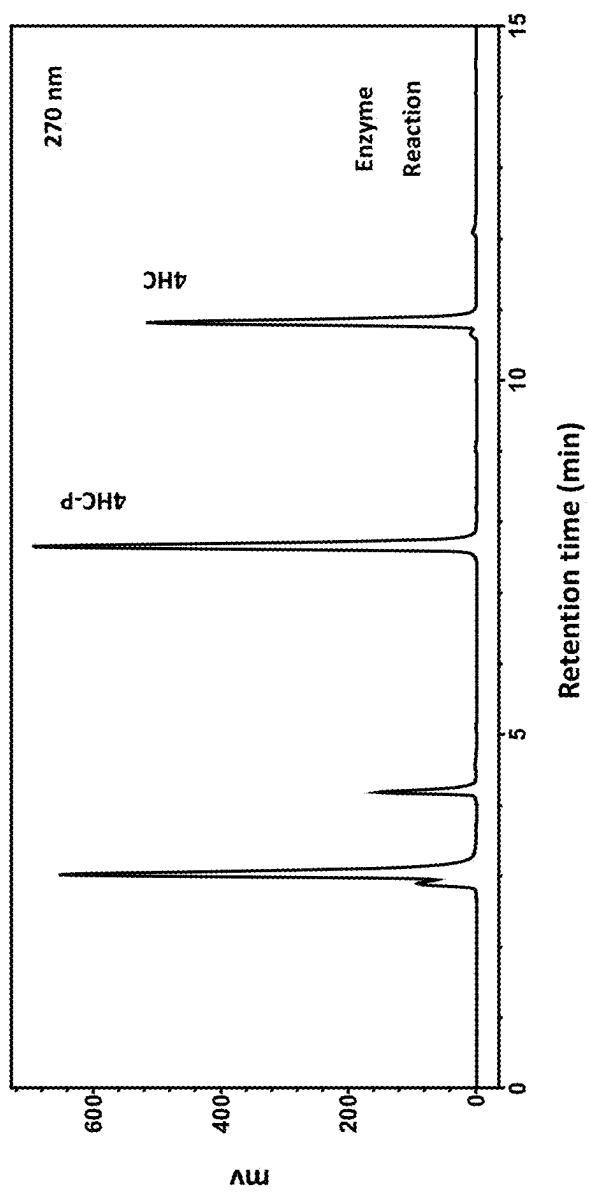
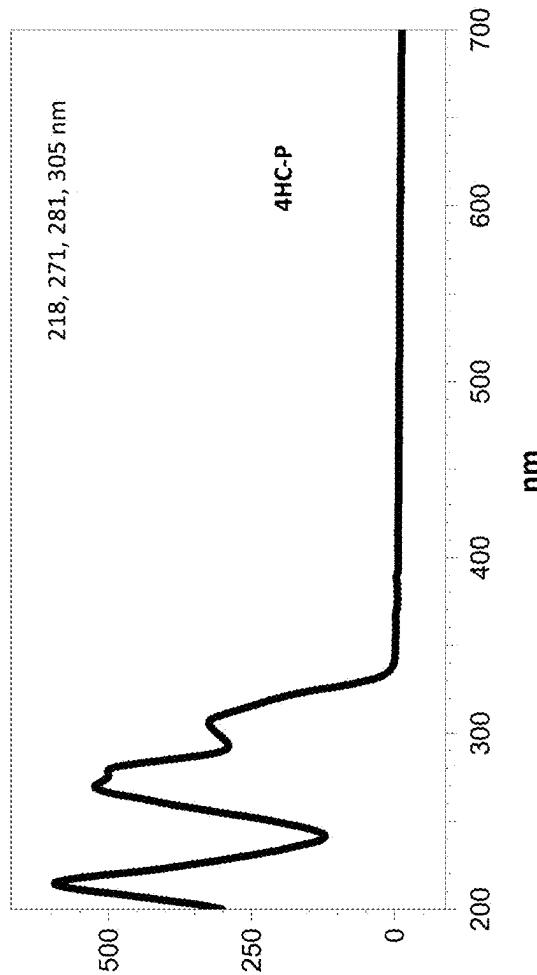


Figure.22(C)



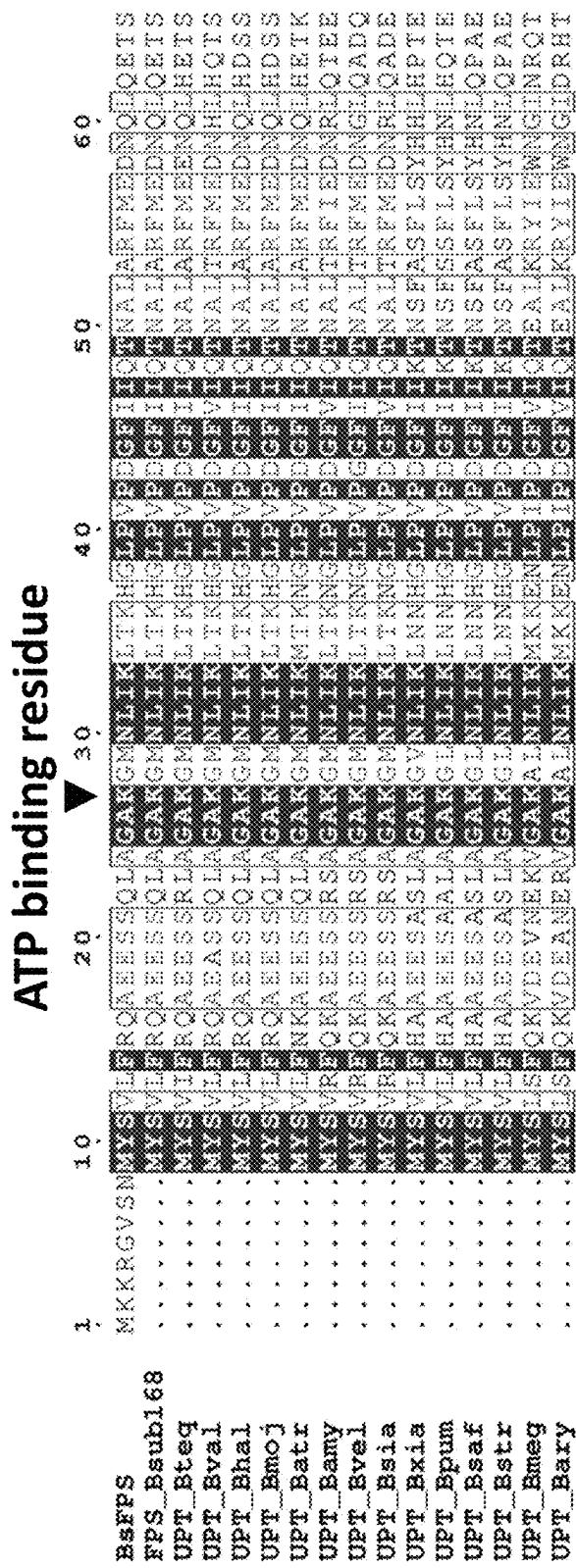


Figure.23(A)

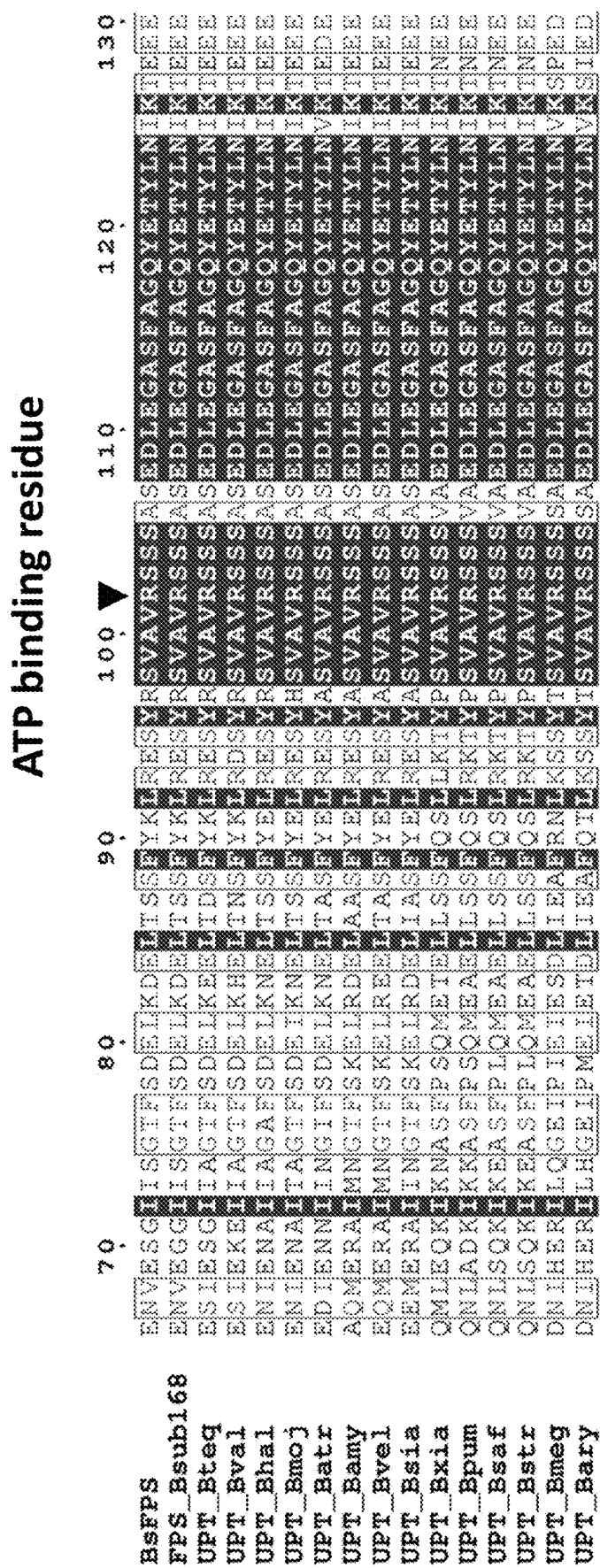


Figure 23(B)

	140	150	160	170	180	190
BSFPS	S	S	S	S	S	S
FPS_Bsub168	S	S	S	S	S	S
UPT_Bteq	E	E	E	E	E	E
UPT_Brai	E	E	E	E	E	E
UPT_Bhal	E	E	E	E	E	E
UPT_Bmoj	E	E	E	E	E	E
UPT_Batr	E	E	E	E	E	E
UPT_Bamy	E	E	E	E	E	E
UPT_Bvel	E	E	E	E	E	E
UPT_Bsia	E	E	E	E	E	E
UPT_Bxia	E	E	E	E	E	E
UPT_Bpum	E	E	E	E	E	E
UPT_Bsaf	E	E	E	E	E	E
UPT_Bstr	E	E	E	E	E	E
UPT_Bmeq	E	E	E	E	E	E
UPT_Bary	E	E	E	E	E	E

Figure.23(C)

Figure.23(D)

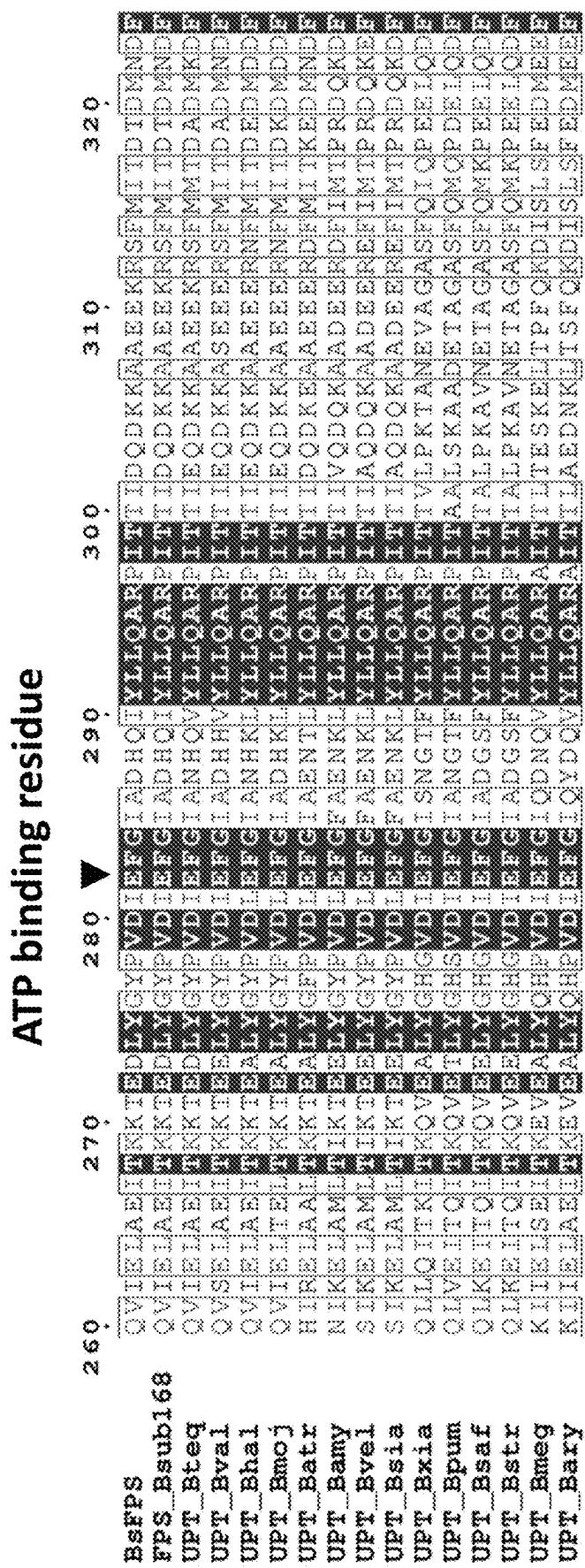


Figure.23(E)

	330	340	350	360	370	380
Bsfps	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
fps_Bsub168	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_Bteq	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_Bval	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_Bhai	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_Bmcj	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_Batt	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_Bamy	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_Bvel	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_Bsia	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_Bxia	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_Bpum	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_BsaF	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_Bstr	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_BmeG	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP
UPt_Bary	LINNE	SI	TECPVSEPA	TECPVSEPA	TECPVSEPA	LYRCHIYSKNOGGOOPP

Figure.23(F)

	3 9 0 .	4 0 0 .	4 1 0 .	4 2 0 .	4 3 0 .	4 4 0 .
BSPS	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
FPS_Bsub168	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Bteq	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Bval	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Bhai	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Bmoj	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Batr	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Bamy	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Bvel	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Bsia	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Exia	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Bpum	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Bsaf	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Bstr	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Bmeg	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR
UPT_Bary	SEHXYDINHITYLPE	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR	RTTPQIACQTEHITR

Figure 23(G)

BSFPS	Bsub168
45.0	45.0
46.0	46.0
47.0	47.0
48.0	48.0
49.0	49.0
50.0	50.0
51.0	51.0

Figure.23(H)

BSERPS	FPS_Bsub168
UPT_Bteq	UPT_Bteq
UPT_Bval	UPT_Bval
UPT_Bhal	UPT_Bhal
UPT_Bmoj	UPT_Bmoj
UPT_Batr	UPT_Batr
UPT_Bamy	UPT_Bamy
UPT_Bvel	UPT_Bvel
UPT_Bsia	UPT_Bsia
UPT_Bxia	UPT_Bxia
UPT_Bpum	UPT_Bpum
UPT_Bsaaf	UPT_Bsaaf
UPT_Bstr	UPT_Bstr
UPT_Bmeg	UPT_Bmeg
UPT_Bary	UPT_Bary

Figure.23(1)

Figure.23(J)

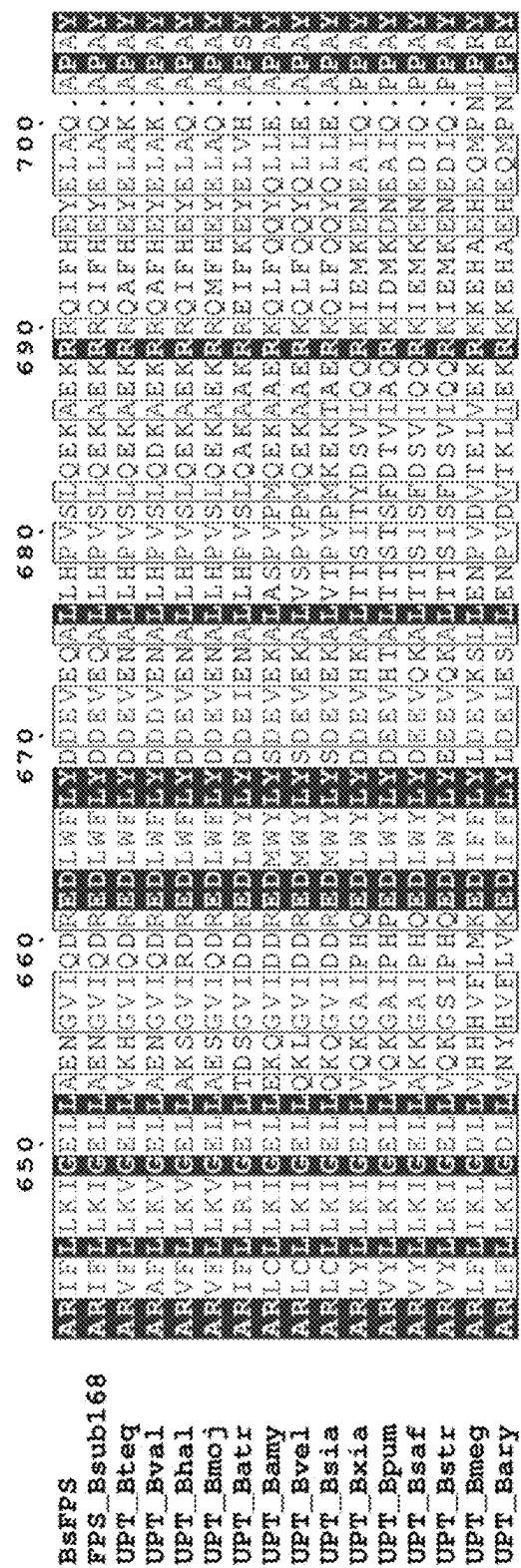


Figure.23(K)



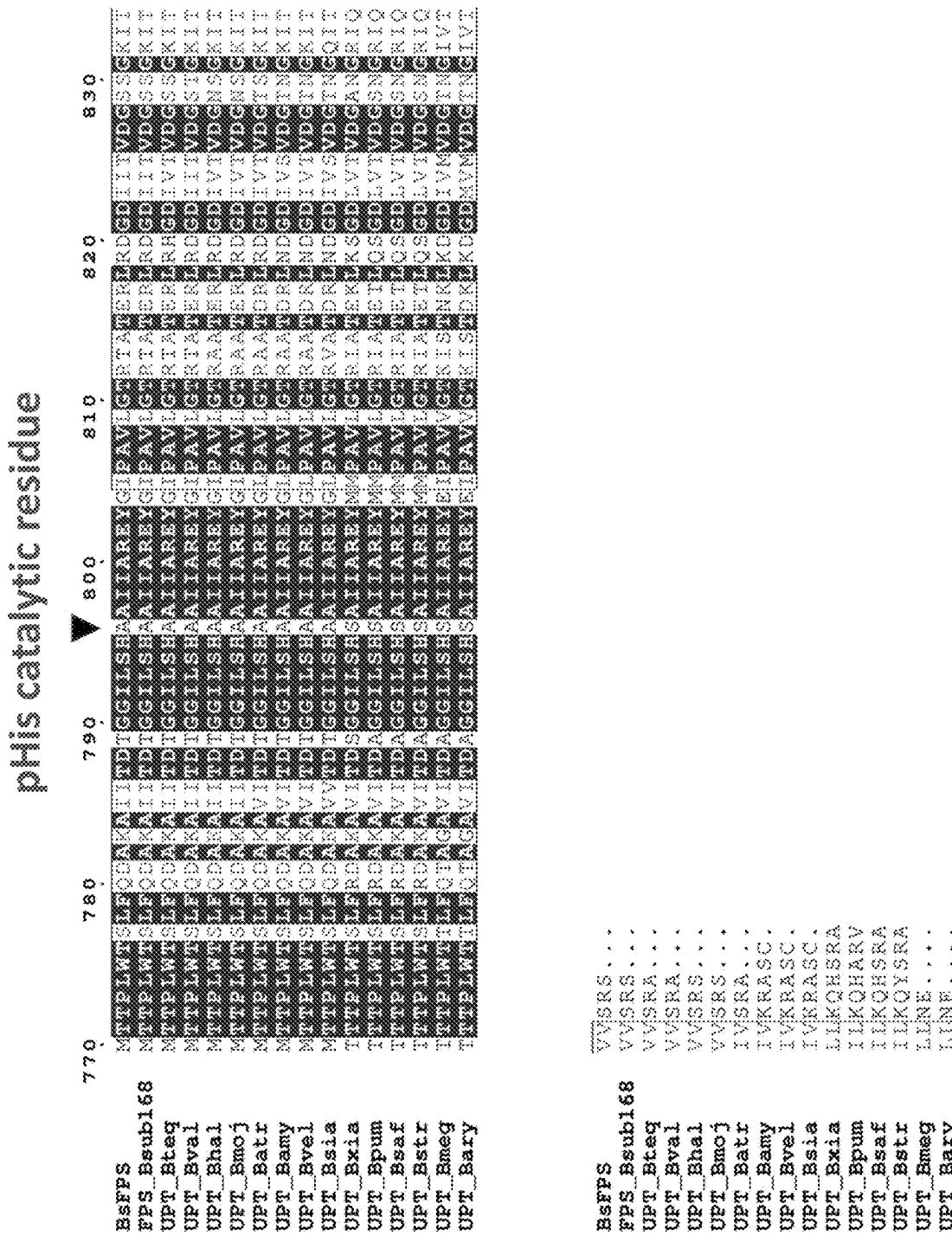


Figure 23(M)

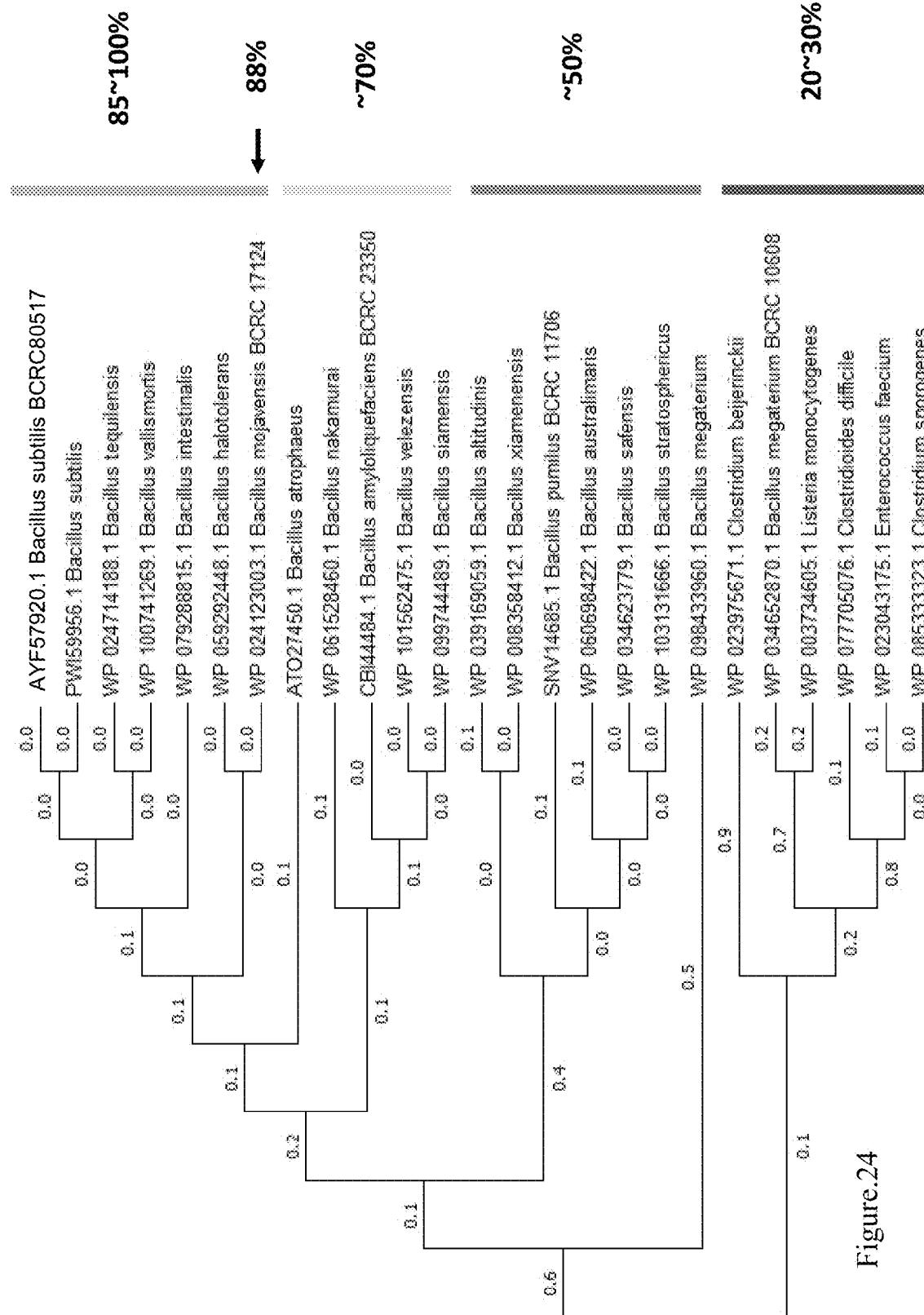


Figure.24

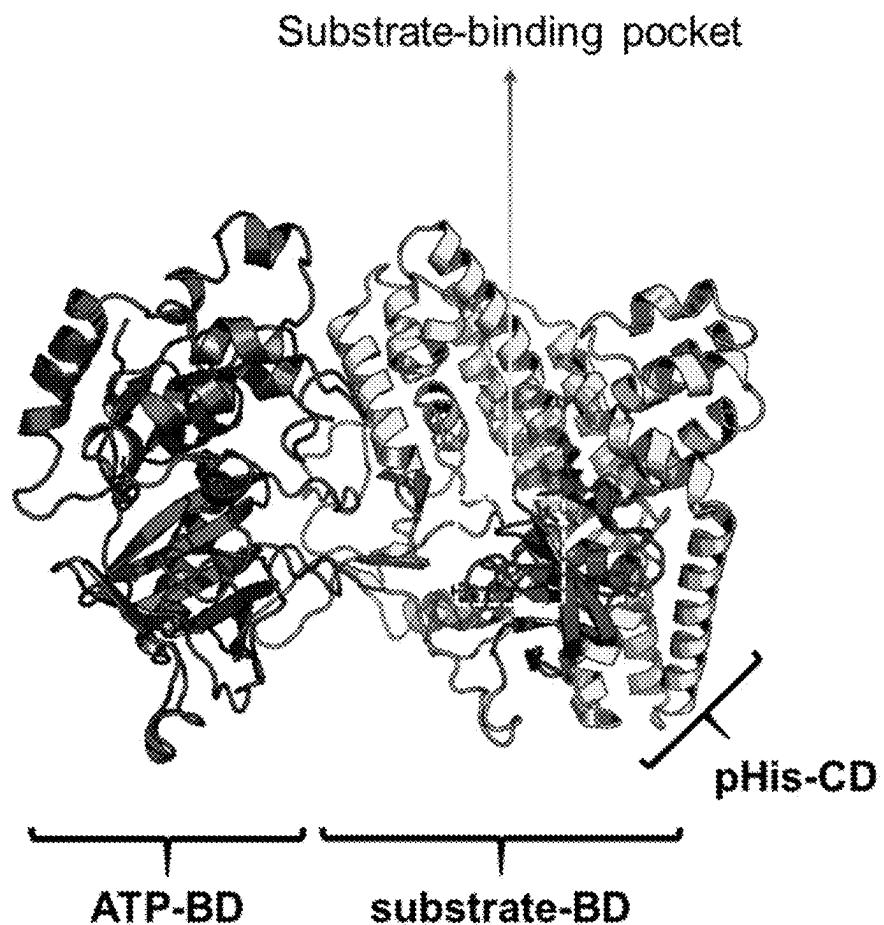


Figure.25

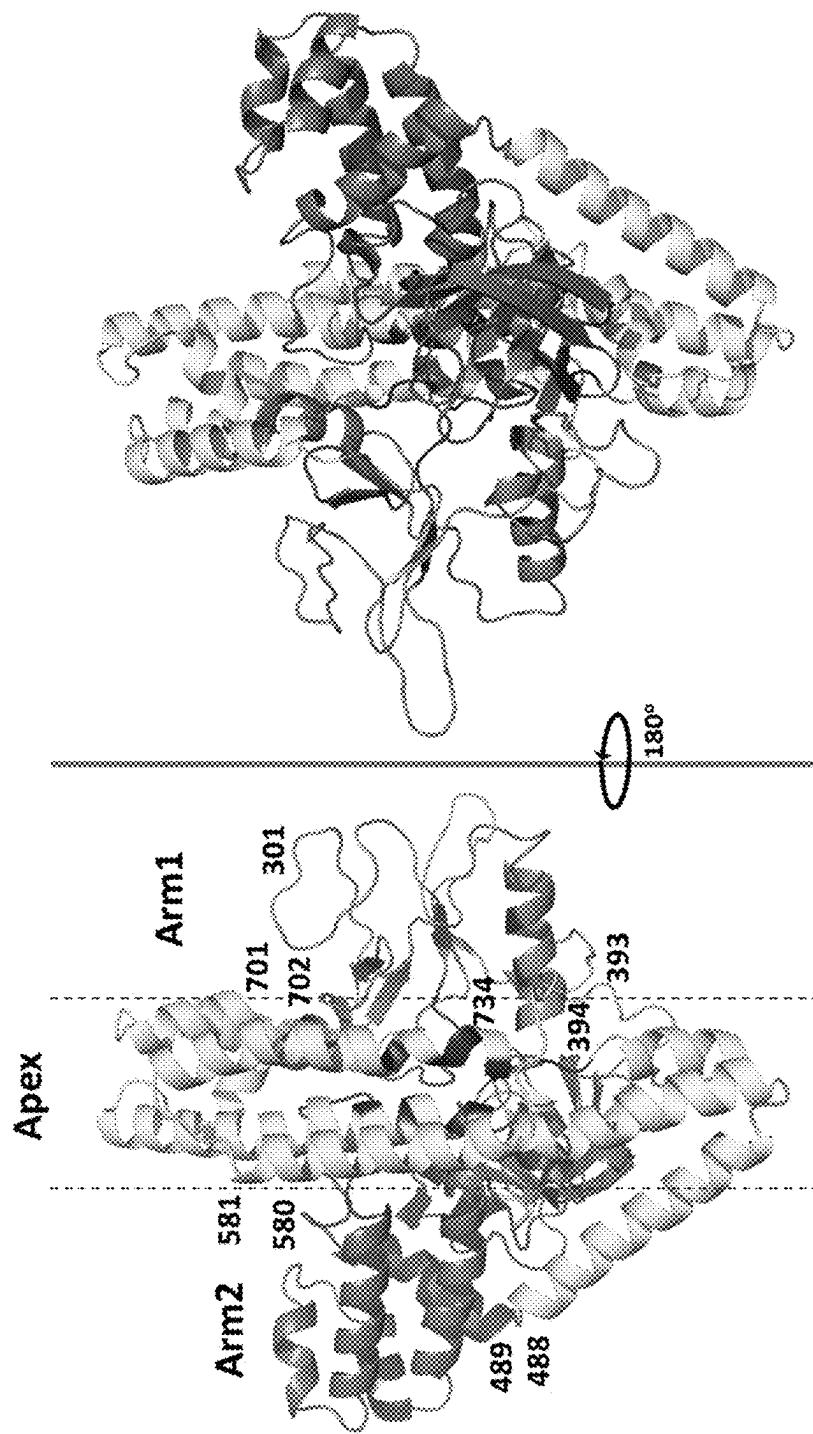


Figure.26

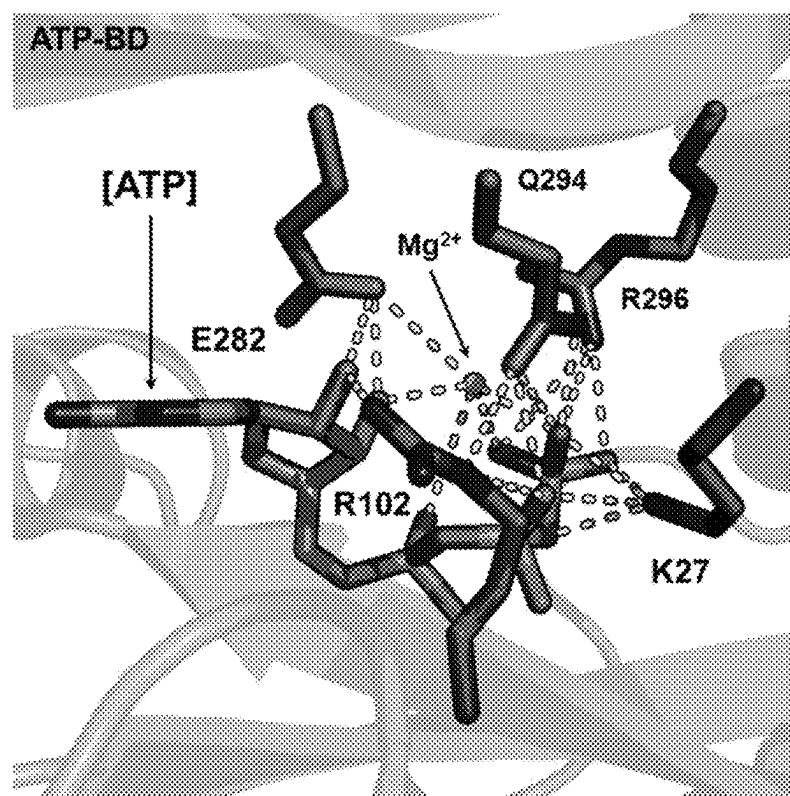


Figure 27(A)

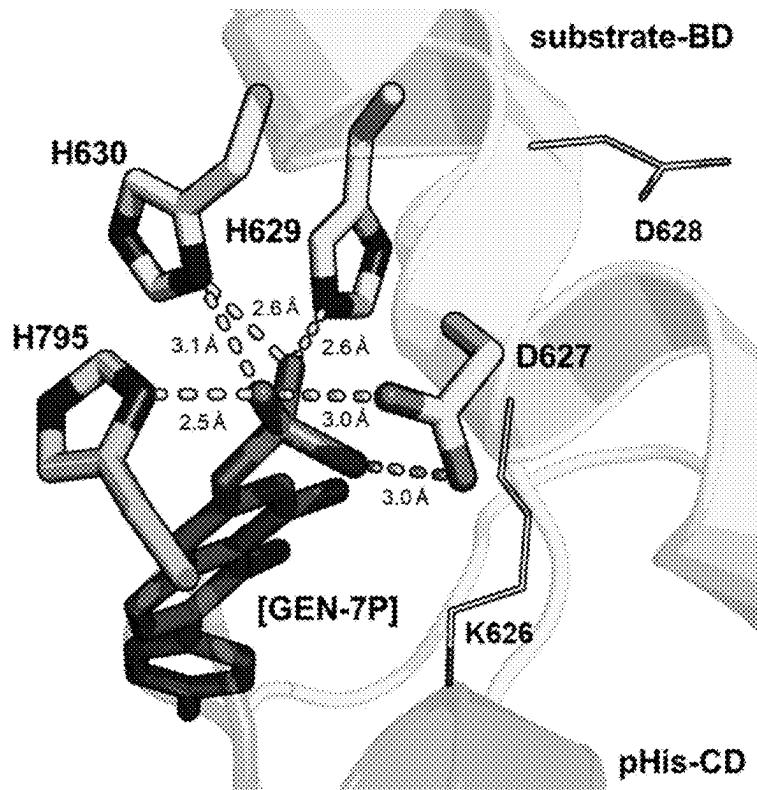


Figure 27(B)

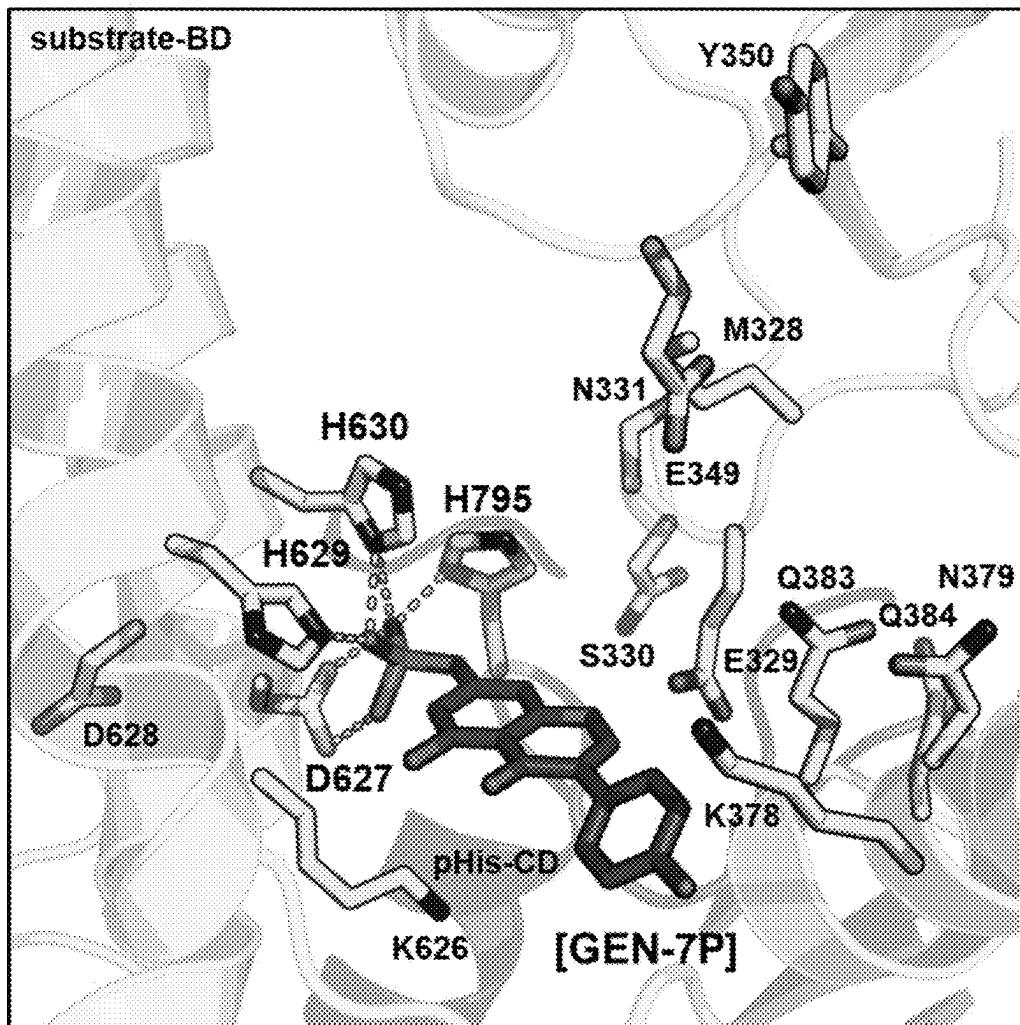


Figure.28

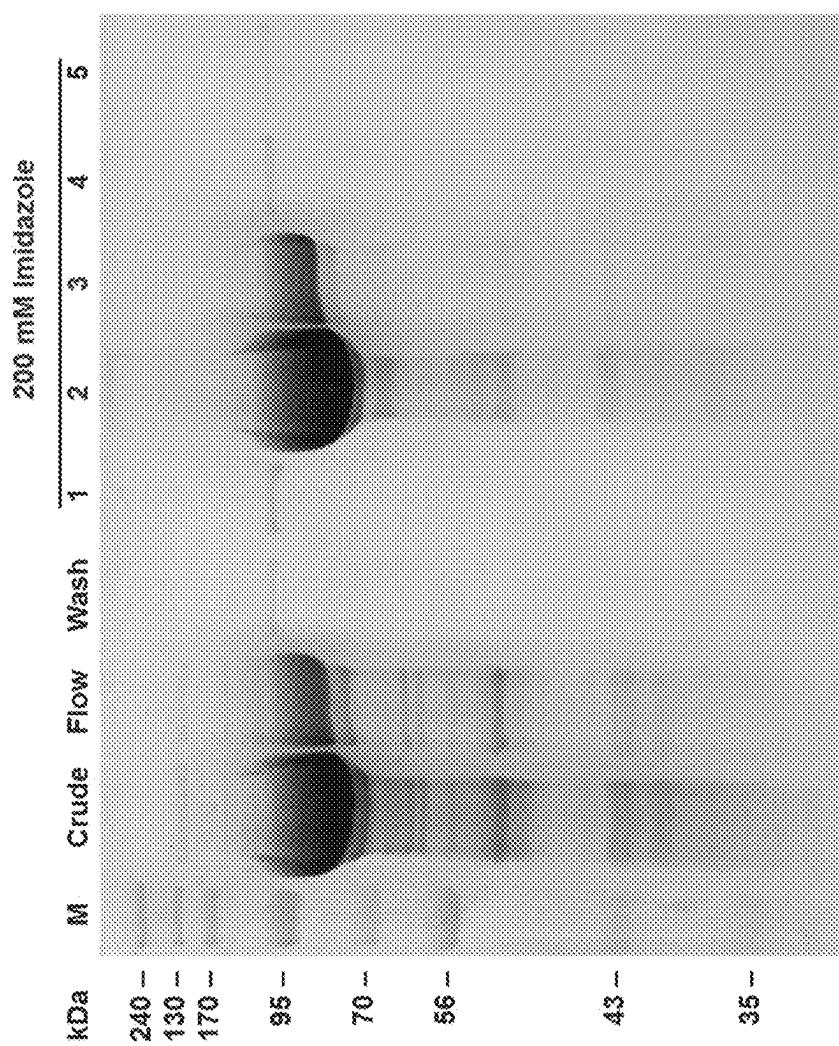


Figure.29

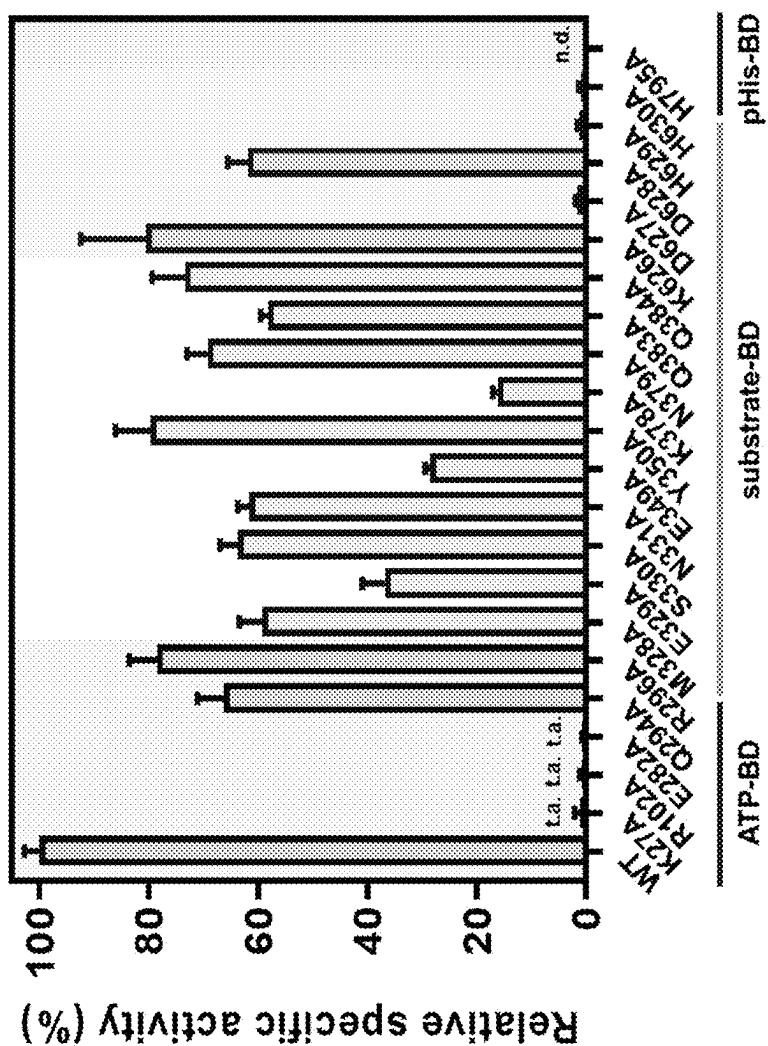


Figure 30

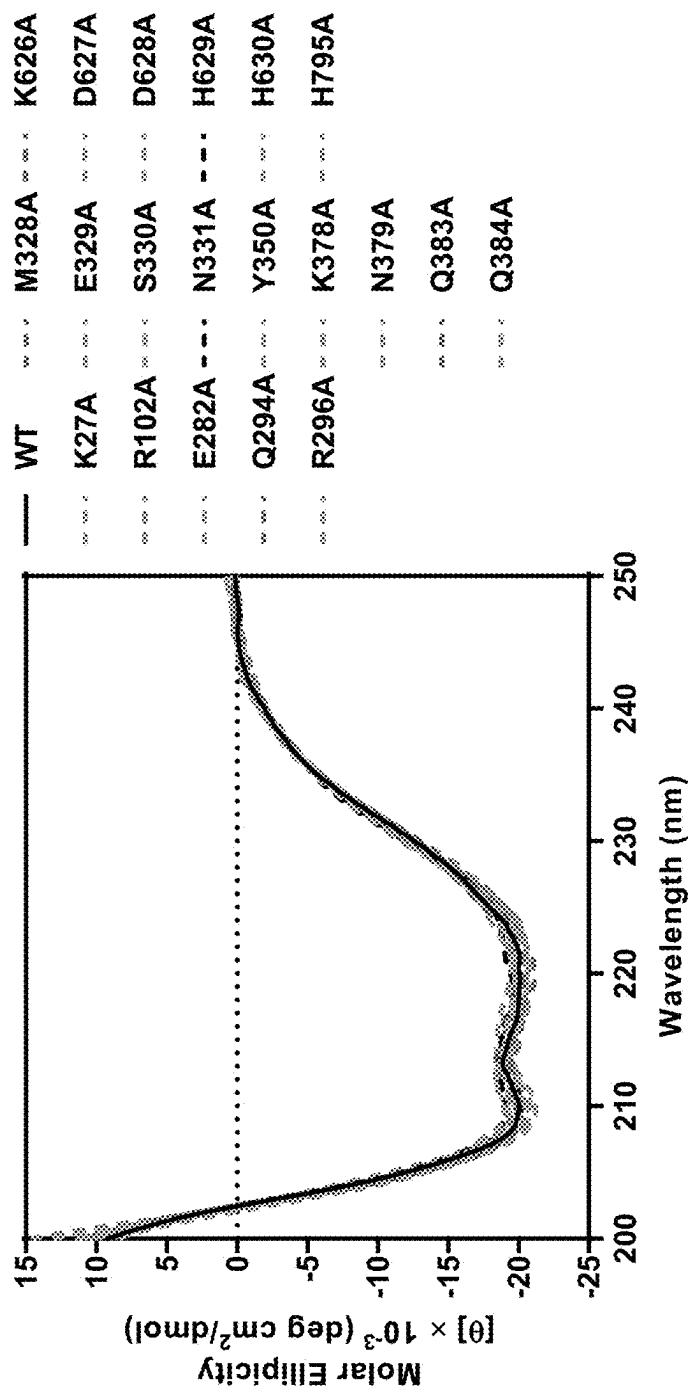


Figure.31

**ISOLATED OR ENGINEERED  
POLYPEPTIDES, MICROORGANISMS AS  
WELL AS METHOD FOR SYNTHESIZING  
POLYPHENOLIC PHYTOCHEMICALS  
PHOSPHATE DERIVATIVE USING THE  
POLYPEPTIDES OR MICROORGANISMS**

**CROSS REFERENCE TO RELATED  
APPLICATIONS**

**[0001]** This application is a Divisional of co-pending application Ser. No. 17/852,836 filed on Jun. 29, 2022, for which priority is claimed under 35 U.S.C. § 120; and this application claims priority of application No. 110136667 filed in Taiwan, R.O.C. on Oct. 1, 2021 under 35 U.S.C. § 119; the entire contents of all of which are hereby incorporated by reference.

**REFERENCE TO AN ELECTRONIC SEQUENCE  
LISTING**

**[0002]** The content of the electronic sequence listing (Substitute Sequence Listing.xml; Size: 50,204 bytes; and Date of Creation: May 1, 2025) is herein incorporated by reference in its entirety.

**BACKGROUND OF THE INVENTION**

1. Technical Field

**[0003]** The present invention relates to an isolated or engineered polypeptide, a microorganism comprising a nucleic acid sequence encoding the polypeptide, and a method for synthesizing a polyphenolic phytochemicals phosphate derivative using the polypeptide or the microorganism. More specifically, said polypeptide has a homologous protein sequence that is more than 70% identical to the polyphenol phosphorylation synthetase (SEQ ID NO: 13).

2. Description of Related Art

**[0004]** Many researches have shown that consumption of polyphenolic phytochemicals helps prevent or delay the occurrence of multimorbidity. It was pointed out in recent studies, however, that polyphenolic phytochemicals are absorbed to a very limited extent. For higher bioavailability, polyphenolic phytochemicals may have their physical and chemical properties improved by structural modification. Currently, the main modification reactions studied in relation to biotransformation of flavonoids are hydroxylation, dehydroxylation, O-methylation, O-demethylation, glycosylation, deglycosylation, hydrogenation, dehydrogenation, C-ring cleavage of the benzo- $\gamma$ -pyrone system, cyclization, and carbonyl reduction.

**[0005]** Cunninghamella, Penicillium, and Aspergillus are the most commonly used microbes in cultures for transformation purposes, in particular *Aspergillus niger*, which can transform flavanone into flavan-4-ol, 2'-hydroxydihydrochalcone, 3-hydroxyflavone, 6-hydroxyflavanone, 4'-hydroxyflavanone, and so on.

**[0006]** Microbes that were used for biotransformation in previous studies also include *Bacillus subtilis* var. *natto*, which typically performs deglycosylation on glycosylated flavonoids, and *Bacillus subtilis* NTU-18, which was found to have high  $\beta$ -glucosidase activity and be capable of highly efficient deglycosylation of glycosylated flavonoids. Moreover, research results have shown that during solid-state

fermentation of black beans with *Bacillus subtilis* BCRC 14715, the glycosylated flavonoid content decreases whereas the deglycosylated flavonoid content increases, and that the resulting black bean natto has a higher percentage of deglycosylated flavonoids out of total flavonoids than the original black beans.

**[0007]** In addition, there have been researches in which *Bacillus subtilis* BCRC 80517 is obtained from commercially available natto products and then used to carry out phosphorylation-based modification on daidzein and genistein in order to produce daidzein 7-O-phosphate (D7P) and genistein 7-O-phosphate (G7P). D7P and G7P are a hundred thousand times as water-soluble as daidzein and genistein, respectively.

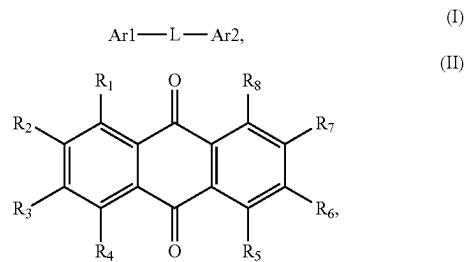
**BRIEF SUMMARY OF THE INVENTION**

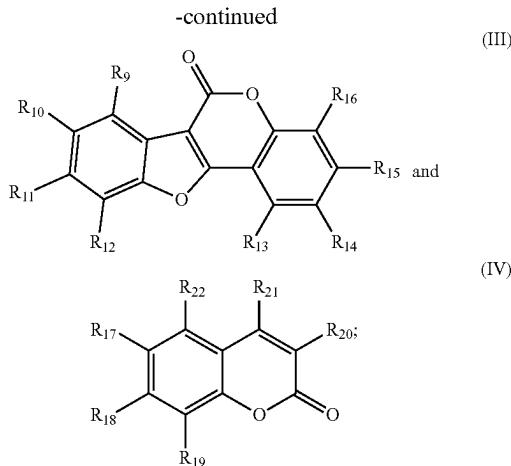
**[0008]** While there have been studies on biotransformation of flavonoids, no literature can be found that relates to modification of polyphenolic phytochemicals by microbial phosphorylation. When the structure of a polyphenolic phytochemical is modified by microbial phosphorylation, the modification conditions are milder than those of chemical modification, and there is no need to use large amounts of chemicals or organic solvents, meaning microbial phosphorylation is a relatively environmentally friendly method for structural modification. The polyphenol phosphorylation phenomenon in the present invention is a novel mode of biotransformation

**[0009]** In view of this, a first aspect of the present invention relates to an isolated or engineered polypeptide comprising a homologous protein sequence that is more than 70% identical to the polyphenol phosphorylation synthetase (SEQ ID NO: 13); wherein said polypeptide comprises a conserved domain which is based on the polyphenol phosphorylation synthetase (SEQ ID NO: 13) and sequentially comprises: an ATP-binding domain comprising active catalytic sites of Lys27, Arg102, and Glu282; a substrate-binding domain comprising a conserved motif of DDHHFYIDAMLDKAR (SEQ ID NO: 14), and comprising active catalytic sites of Asp627, His629, and His630; and a phosphorylated histidine catalytic domain comprising His795.

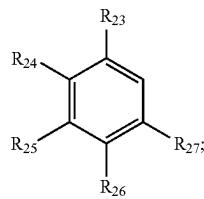
**[0010]** Preferably, the polypeptide catalyzes a substrate to its phosphate derivative, and said substrate is a polyphenolic phytochemical.

**[0011]** Preferably, the substrate is selected from the group consisting of the following formulas:

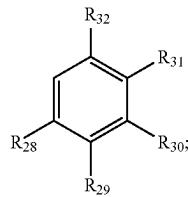




[0012] wherein Ar1 is an aryl group of the following formula:



[0013] Ar2 is an aryl group of the following formula:



[0014] L is a linking group comprising 3 to 7 backbone carbon atoms forming a chain linking Ar1 and Ar2 as the case may be, wherein L comprises at least one of a double bond, a carbonyl group and a hydroxyl group;

[0015] R1 to R8 are respectively H, (C1-C5)alkyl group, hydroxyl group, OR33, OCH2OR34, OCOR35, COR36, CO2R37, OCH2COOR38, OCH2 (OR39)2, OC=ONHR40, halogen, nitro, amino, NR41R42, cyano group, mercapto group, SR43, S(O)qR44, (C1-C5)chloroalkyl group, (C1-C5)haloalkoxy group, (C2-C6)alkenyl group, (C2-C6)alkynyl group, (C3-C10) cycloalkyl group, (C6-C11)phenyl group or (C7-C12) benzyl group, wherein q is an integral of 1 to 3, and at least one of R1 to R8 is a hydroxyl group;

[0016] R9 to R16 are respectively H, (C1-C5)alkyl group, hydroxyl group, OR33, OCH2OR34, OCOR35, COR36, CO2R37, OCH2COOR38, OCH2 (OR39)2, OC=ONHR40, halogen, nitro, amino, NR41R42, cyano group, mercapto group, SR43, S(O)qR44, (C1-C5)chloroalkyl group, (C1-C5)haloalkoxy group, (C2-

C6)alkenyl group, (C2-C6)alkynyl group, (C3-C10) cycloalkyl group, (C6-C11)phenyl group or (C7-C12) benzyl group, wherein q is an integral of 1 to 3, and at least one of R9 to R16 is a hydroxyl group;

[0017] R17 to R22 are respectively H, methoxy group or hydroxyl group, and at least one of R17 to R22 is a hydroxyl group, or R20 and R21, R17 and R18, R17 and R22, R18 and R19 or their combination are fused to form a (C3-C6)cycloalkyl group with hydroxyl group or a (C6-C10)aryl group with hydroxyl group;

[0018] R23 to R27 are respectively H, methoxy group or hydroxyl group, and at least one of R23 to R27 is a hydroxyl group;

[0019] R28 to R32 are respectively H, methoxy group or hydroxyl group, and at least one of R28 to R32 is a hydroxyl group;

[0020] R33 to R34 are respectively (C1-C5)alkyl group, (C1-C5)haloalkoxy group, (C2-C6)alkenyl group, (C2-C6)alkynyl group, (C6-C11)phenyl group or (C7-C12) benzyl group;

[0021] R35 is (C1-C5)alkyl group, (C1-C5)haloalkoxy group, (C6-C11)phenyl group or (C7-C12)benzyl group;

[0022] R36 is (C1-C5)alkyl group, (C1-C5)haloalkoxy group, (C2-C6)alkenyl group, (C2-C6)alkynyl group, (C6-C11)phenyl group or (C7-C12)benzyl group; R37 to R40 are respectively (C1-C5)alkyl group or (C1-C5) haloalkoxy group; R41 and R42 are respectively H, (C1-C5)alkyl group or (C1-C5)haloalkoxy group, one of which is H and the other is not H;

[0023] R43 and R44 are respectively H, (C1-C5)alkyl group or (C1-C5)haloalkoxy group.

[0024] Preferably, the substrate is a natural or modified curcuminoid, anthraquinones, chalcone, stilbenoid, coumestan or coumarin.

[0025] Preferably, the curcuminoid is a natural or modified curcumin, bisdemethoxycurcumin, tetrahydrocurcumin or octahydrocurcumin; the anthraquinone is a natural or modified emodin, obtusifolin or aloë-emodin; the chalcone is a natural or modified phloretin, isoliquiritigenin or flavokawain A; the stilbenoid is a natural or modified resveratrol, pterostilbene, piceatannol or oxyresveratrol; the coumestan is a natural or modified coumestrol, wedelolactone or demethylwedelolactone; the coumarin is a natural or modified umbelliferone or 4-hydroxycoumarin.

[0026] Another aspect of the present invention relates to a microorganism comprising a nucleic acid sequence encoding said polypeptide.

[0027] Preferably, the nucleic acid sequence is derived from *Bacillus subtilis*, *Bacillus halotolerans*, *Bacillus atropphaeus*, *Bacillus mojavensis*, *Bacillus velezensis* or *Bacillus amyloliquefaciens*.

[0028] Another aspect of the present invention relates to a method for synthesizing a polyphenolic phytochemicals phosphate derivative, comprising: exposing a polyphenolic phytochemical to said polypeptide or to a microorganism comprising a nucleic acid sequence encoding said polypeptide for converting the polyphenol phytochemical to its phosphate derivatives.

[0029] Preferably, the polyphenolic phytochemical is a natural or modified curcuminoid, anthraquinone, chalcone, stilbenoid, coumestan or coumarin.

[0030] As stated above, the present invention ascertains important catalytic sites of a polypeptide so that when the

polypeptide or a microbe having the nucleic acid sequence coding for the polypeptide is used to synthesize a polyphenolic phytochemical phosphate, the success rate of polyphenolic phytochemical phosphorylation or the yield of the synthetic polyphenolic phytochemical phosphate derivative will be increased in comparison with that of the prior art.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0031] The embodiments of the present invention are described by way of example only, with reference to the accompanying drawings, wherein:

[0032] FIG. 1 shows percent conversion rate of each polyphenol by the recombinant PPSs when using ATP as phosphate donor; the patterns of the bar graphs represent the ratio of phosphorylated products at different O-position in the total product yield of each polyphenol, and the trace products (yield <2%) are not counted; experiments were performed in triplicate, and the S.D. is shown.

[0033] FIGS. 2(A) to 2(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and curcumin (CUR): (A) the structure of the curcumin; (B) HPLC chromatogram of curcumin before reaction; (C) The HPLC-UV/Vis spectrum (430 nm) of the curcumin phosphate (CUR-P) produced after the recombinant PPSs reacted with curcumin for 1 hour at 40° C. and pH 7.8.

[0034] FIGS. 3(A) to 3(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and bisdemethoxycurcumin (BDMC): (A) the structure of the bisdemethoxycurcumin; (B) HPLC chromatogram of bisdemethoxycurcumin before reaction; (C) The HPLC-UV/Vis spectrum (430 nm) of the bisdemethoxycurcumin phosphate (BDMC-P) produced after the recombinant PPSs reacted with bisdemethoxycurcumin for 1 hour at 40° C. and pH 7.8.

[0035] FIGS. 4(A) to 4(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and tetrahydrocurcumin (THC): (A) the structure of the tetrahydrocurcumin; (B) HPLC chromatogram of tetrahydrocurcumin before reaction; (C) The HPLC-UV/Vis spectrum (280 nm) of the tetrahydrocurcumin phosphate (THC-P) produced after the recombinant PPSs reacted with tetrahydrocurcumin for 1 hour at 40° C. and pH 7.8.

[0036] FIGS. 5(A) to 5(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and octahydrocurcumin (OHC): (A) the structure of the octahydrocurcumin; (B) HPLC chromatogram of octahydrocurcumin before reaction; (C) The HPLC-UV/Vis spectrum (280 nm) of the octahydrocurcumin phosphate (OHC-P) produced after the recombinant PPSs reacted with octahydrocurcumin for 1 hour at 40° C. and pH 7.8.

[0037] FIGS. 6(A) to 6(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and emodin (EMD): (A) the structure of the emodin; (B) HPLC chromatogram of emodin before reaction; (C) The HPLC-UV/Vis spectrum (260 nm) of the emodin phosphate (EMD-P) produced after the recombinant PPSs reacted with emodin for 1 hour at 40° C. and pH 7.8.

[0038] FIGS. 7(A) to 7(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and obtusifolin (OBF): (A) the structure of the obtusifolin; (B) HPLC chromatogram of obtusifolin before reaction; (C) The HPLC-UV/Vis spectrum (260 nm) of the obtusifolin phosphate (OBF-P) produced after the recombinant PPSs reacted with obtusifolin for 1 hour at 40° C. and pH 7.8.

[0039] FIGS. 8(A) to 8(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and aloe-emodin (ALE): (A) the structure of the aloe-emodin; (B) HPLC chromatogram of aloe-emodin before reaction; (C) The HPLC-UV/Vis spectrum (260 nm) of the aloe-emodin phosphate (ALE-P) produced after the recombinant PPSs reacted with aloe-emodin for 1 hour at 40° C. and pH 7.8.

[0040] FIGS. 9(A) to 9(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and phloretin (PHL): (A) the structure of the phloretin; (B) HPLC chromatogram of phloretin before reaction; (C) The HPLC-UV/Vis spectrum (310 nm) of the phloretin phosphate (PHL-P) produced after the recombinant PPSs reacted with phloretin for 1 hour at 40° C. and pH 7.8.

[0041] FIGS. 10(A) to 10(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and isoliquiritigenin (ISL): (A) the structure of the isoliquiritigenin; (B) HPLC chromatogram of isoliquiritigenin before reaction; (C) The HPLC-UV/Vis spectrum (310 nm) of the isoliquiritigenin phosphate (ISL-P) produced after the recombinant PPSs reacted with isoliquiritigenin for 1 hour at 40° C. and pH 7.8.

[0042] FIGS. 11(A) to 11(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and flavokawain A (FKA): (A) the structure of the flavokawain A; (B) HPLC chromatogram of flavokawain A before reaction; (C) The HPLC-UV/Vis spectrum (280 nm) of the flavokawain A phosphate (FKA-P) produced after the recombinant PPSs reacted with flavokawain A for 1 hour at 40° C. and pH 7.8.

[0043] FIGS. 12(A) to 12(D) show a chemical analysis spectrum of the reaction of the recombinant PPSs and resveratrol (RES): HPLC chromatogram of resveratrol before (A) and after (B) the reaction of resveratrol with the recombinant PPSs for 2 hours at 40° C., pH 7.8; (C) The HPLC spectrum of (C) the RES-3P and (D) RES-4P standards purified from semi-preparative HPLC.

[0044] FIGS. 13(A) to 13(D) show a NMR spectra of resveratrol 3-O-phosphate: (A) the structure of resveratrol 3-O-phosphate, (B) 1H (in DMSO-d<sub>6</sub>, 500 MHz), (C) 2D HMQC 1H/31P (TPP in acetone-d<sub>6</sub>, 202 MHz for 31P NMR), (D) 13C (in DMSO-d<sub>6</sub>, 125 MHz) NMR spectra of resveratrol 3-O-phosphate.

[0045] FIGS. 14(A) to 14(D) show a NMR spectra of resveratrol 4'-O-phosphate: (A) the structure of resveratrol 4'-O-phosphate, (B) 1H (in DMSO-d<sub>6</sub>, 500 MHz), (C) 2D HMQC 1H/31P (TPP in acetone-d<sub>6</sub>, 202 MHz for 31P NMR), (D) 13C (in DMSO-d<sub>6</sub>, 125 MHz) NMR spectra of resveratrol 4'-O-phosphate.

[0046] FIGS. 15(A) to 15(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and pterostilbene (PTER): (A) the structure of the pterostilbene; (B) HPLC chromatogram of pterostilbene before reaction; (C) The HPLC-UV/Vis spectrum (280 nm) of the pterostilbene phosphate (PTER-P) produced after the recombinant PPSs reacted with pterostilbene for 1 hour at 40° C. and pH 7.8.

[0047] FIGS. 16(A) to 16(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and piceatannol (PCT): (A) the structure of the piceatannol; (B) HPLC chromatogram of piceatannol before reaction; (C) The HPLC-UV/Vis spectrum (280 nm) of the piceatannol

phosphates (PCT-P1, PCT-P2) produced after the recombinant PPSs reacted with piceatannol for 1 hour at 40° C. and pH 7.8.

[0048] FIGS. 17(A) to 17(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and oxyresveratrol (OXY): (A) the structure of the oxyresveratrol; (B) HPLC chromatogram of oxyresveratrol before reaction; (C) The HPLC-UV/Vis spectrum (280 nm) of the oxyresveratrol phosphates (OXY-P1, OXY-P2, OXY-P3) produced after the recombinant PPSs reacted with oxyresveratrol for 1 hour at 40° C. and pH 7.8.

[0049] FIGS. 18(A) to 18(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and coumestrol (CUM): (A) the structure of the coumestrol; (B) HPLC chromatogram of coumestrol before reaction; (C) The HPLC-UV/Vis spectrum (254 nm) of the coumestrol phosphate (CUM-P) produced after the recombinant PPSs reacted with coumestrol for 1 hour at 40° C. and pH 7.8.

[0050] FIGS. 19(A) to 19(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and wedelolactone (WDL): (A) the structure of the wedelolactone; (B) HPLC chromatogram of wedelolactone before reaction; (C) The HPLC-UV/Vis spectrum (254 nm) of the wedelolactone phosphates (WDL-P1, WDL-P2) produced after the recombinant PPSs reacted with wedelolactone for 1 hour at 40° C. and pH 7.8.

[0051] FIGS. 20(A) to 20(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and demethylwedelolactone (DMWDL): (A) the structure of the demethylwedelolactone; (B) HPLC chromatogram of demethylwedelolactone before reaction; (C) The HPLC-UV/Vis spectrum (254 nm) of the demethylwedelolactone phosphates (DMWDL-P1, DMWDL-P2) produced after the recombinant PPSs reacted with demethylwedelolactone for 1 hour at 40° C. and pH 7.8.

[0052] FIGS. 21(A) to 21(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and umbelliferone (UMB): (A) the structure of the umbelliferone; (B) HPLC chromatogram of umbelliferone before reaction; (C) The HPLC-UV/Vis spectrum (320 nm) of the umbelliferone phosphate (UMB-P) produced after the recombinant PPSs reacted with umbelliferone for 1 hour at 40° C. and pH 7.8.

[0053] FIGS. 22(A) to 22(C) show a chemical analysis spectrum of the reaction of the recombinant PPSs and 4-hydroxycoumarin (4HC): (A) the structure of the 4-hydroxycoumarin; (B) HPLC chromatogram of 4-hydroxycoumarin before reaction; (C) The HPLC-UV/Vis spectrum (270 nm) of the 4-hydroxycoumarin phosphate (4HC-P) produced after the recombinant PPSs reacted with 4-hydroxycoumarin for 1 hour at 40° C. and pH 7.8.

[0054] FIGS. 23(A) to 23(M) show multiple sequence alignments of BsPPS with orthologs from different *Bacillus* species; wherein the sequences are listed as follows: BsFPS (SEQ ID NO: 15), FPS\_Bsub168 (SEQ ID NO: 16), UPT\_Bteq (SEQ ID NO: 17), UPT\_Bval (SEQ ID NO: 18), UPT\_Bhal (SEQ ID NO: 19), FPS\_Bmoj (SEQ ID NO: 20), UPT\_Batr (SEQ ID NO: 21), FPS\_Bamy (SEQ ID NO: 22), UPT\_Bvel (SEQ ID NO: 23), UPT\_Bsia (SEQ ID NO: 24), UPT\_Bxia (SEQ ID NO: 25), UPT\_Bpum (SEQ ID NO: 26), UPT\_Bsaf (SEQ ID NO: 27), UPT\_Bstr (SEQ ID NO: 28), UPT\_Bmeg (SEQ ID NO: 29), UPT\_Bary (SEQ ID NO: 30).

[0055] FIG. 24 shows a phylogenetic tree of PPS homologous protein sequences.

[0056] FIG. 25 shows a simulated tertiary structure for predicting PPS using LmRPH (PDB entry, 5FBU) as a template.

[0057] FIG. 26 shows a simulated structure of the substrate-binding region of PPS.

[0058] FIGS. 27(A) and (27(B) show: (A) Active sites of PPS homology model that dock with Mg<sup>2+</sup>/ATP; (B) PPS homology model and catalytic sites of phosphorylation active region thereof that dock with substrates.

[0059] FIG. 28 shows important catalytic sites predicted by the outward extension of the PPS phosphorylation active region.

[0060] FIG. 29 shows an analysis of the protein purified by Histrap™ HP column by SDS-PAGE (M: protein molecular weight; PPS eluate (buffer solution containing 20 mM imidazole)).

[0061] FIG. 30 shows the relative specific activity of the PPS protein mutant strains.

[0062] FIG. 31 shows a far-UV circular dichroism spectrum of the PPS protein of interest and its mutant strain; the presented curve is smoothed by nonlinear regression.

#### DETAILED DESCRIPTION OF THE INVENTION

[0063] The detailed description and technical content of the present invention are now described as follows in conjunction with the drawings. Furthermore, the drawings in the present invention are not necessarily drawn according to the actual scale for the convenience of description. These drawings and their scales are not intended to limit the scope of the present invention.

[0064] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The following terms used throughout this application shall have the following meanings.

[0065] “Or” means “and/or” unless stated otherwise. “Comprising” means not excluding the presence or addition of one or more other components, steps, operations or elements to the described component, step, operation or element, respectively. The terms “comprising”, “including”, “containing”, and “having” as used herein are interchangeable and not limiting. As used herein and in the appended claims, the singular forms “a” and “the” include plural referents unless the context otherwise dictates. For example, the terms “a”, “the”, “one or more” and “at least one” are used interchangeably herein.

[0066] The term “gene” means a genetic unit that occupies a specific locus on a chromosome and consists of transcriptional and/or translational regulatory sequences and/or coding regions and/or non-translated sequences (i.e., introns, 5' and 3' non-translated sequence). References to “mutations” or “deletions” in relation to a gene generally refer to changes or alterations in a gene that result in reduced or no expression of the encoded gene product or render the gene product non-functional or reduced in function compared to a wild-type gene product. Examples of such alterations include nucleotide substitutions, deletions or additions, in whole or in part, to the coding or regulatory sequences of the target gene that disrupt, eliminate, down-regulate or significantly reduce the performance of the polypeptide encoded by the gene, whether at the transcriptional or translational level. In

certain aspects, the targeted gene can be rendered “non-functional” by changes or mutations at the nucleotide level that alter the amino acid sequence of the encoded polypeptide, allowing the modified polypeptide to behave, but have reduced function or activity relative to one or more enzymatic activities, whether by altering the active site of the polypeptide, its cellular localization, its stability, or other functional characteristics as will be apparent to those skilled in the art.

[0067] The terms “polypeptide,” “polypeptide fragment,” “peptide,” and “protein” are used interchangeably herein to refer to polymers of amino acid residues, and variants and synthetic analogs thereof. Accordingly, these terms apply to amino acid polymers in which one or more amino acid residues are synthetic non-naturally occurring amino acids, such as chemical analogs corresponding to naturally occurring amino acids. In certain aspects, polypeptides can include enzymatic polypeptides (or “enzymes”), which typically catalyze (i.e. increasing the rate of) various chemical reactions.

[0068] “Isolated”, as used herein, means that have been (1) separated (whether in nature and/or in an experimental context) from at least some of the components with which they were originally produced, and/or (2) engineered, produced, prepared and/or manufactured by humans. In some embodiments, a substance is “pure” if it is substantially free of other components. In some embodiments, as understood by those skilled in the art, a substance may still be considered “isolated” or even “pure” after being combined with certain other components such as one or more carriers or excipients (e.g. buffers, solvents, water, etc.); in such embodiments, the isolation or purity percentage of the material is calculated without the inclusion of such carriers or excipients. By way of example only, in some embodiments, a biopolymer such as a polypeptide or polynucleotide that occurs in nature is considered “isolated” when a) its source or origin is not the same as that found in nature in its native state associated with some or all of the components accompanied; b) it is substantially free of other polypeptides or nucleic acids from the same species as the species in which it is produced in nature; or c) expressed by cells of a species not in which it is produced in nature or other expression system or otherwise associated with components from said cell or other expression system. Thus, for example, in some embodiments, a polypeptide that is chemically synthesized or synthesized in a cell system different from the cell in which it is produced in nature is considered an “isolated” polypeptide. Alternatively or additionally, in some embodiments, a polypeptide that has been subjected to one or more purification techniques may be considered an “isolated” polypeptide to the extent that it has been separated from other components that: a) are associated in nature and/or b) are associated with which it was originally produced.

[0069] “Conserved”, as used herein, refers to the situation in biology that is similar or identical within a nucleic acid sequence, protein sequence, protein structure or polysaccharide sequence; it may occur between species, or between different molecules arising from the same organism. From an evolutionary point of view, it means a state in which a particular sequence continues to be preserved during the process of speciation.

[0070] The term “modified” does not necessarily mean that a nucleotide/amino acid analog or a non-natural nucleo-

tide/amino acid is obtained by directly altering the natural nucleotides/amino acids, but that nucleotide/amino acid analogs or non-natural nucleotides/amino acids differ from natural nucleotides/amino acids. In some embodiments, the modification comprises chemical modification; when the embodiment involves genetic modification of microorganisms, typical strain development and/or molecular genetic techniques can be used to achieve the effect.

[0071] The present invention relates to an isolated or engineered polypeptide and a microorganism containing said polypeptide, the polypeptide comprises a homologous protein sequence that is more than 70% identical to the polyphenol phosphorylation synthetase (SEQ ID NO: 13); wherein said polypeptide comprises a conserved domain which is based on the polyphenol phosphorylation synthetase (SEQ ID NO: 13) and sequentially comprises: an ATP-binding domain comprising active catalytic sites of Lys27, Arg102, and Glu282; a substrate-binding domain comprising a conserved motif of DDHHFYIDAMLDKAR (SEQ ID NO: 14), and comprising active catalytic sites of Asp627, His629, and His630; and a phosphorylated histidine catalytic domain comprising His795.

[0072] According to some embodiments of the present invention, the degree of identity between the amino acid sequence of the substrate binding domain and the amino acid sequence of SEQ ID NO: 1 is more than 60%; preferably at least 75%, more preferably at least 80%, even more preferably at least 85%; for example, the degree of identity is preferably more than 70%, more than 71%, more than 72%, more than 73%, more than 74%, more than 75%, More than 76%, more than 77%, more than 78%, more than 79%, more than 80%, more than 85%, more than 90%, more than 95% or more than 99%, etc. As used herein, “identity” of an amino acid sequence refers to the degree to which two sequences are mutually indistinguishable, and “similarity” refers to the same ratio and/or retention ratio between the two sequences. Those of ordinary skill in the art to which the present invention pertains should understand that the long-chain amino acids of polypeptides and proteins are only partially functional in their amino acid sequences, which are called functional motifs. Proteins have the same function when they have the same functional motif; in general, when the amino acid sequence of a polypeptide or protein is at least 40% identical, it has the same function (refer to How Proteins Work, Williamson, 2011). After alignment with homologous proteins or polypeptides, the amino acid sequence of the substrate-binding domain can be at least 60% identical to SEQ ID NO: 1, preferably at least 75%, more preferably at least 80%, even more preferably at least 85% identical amino acid sequences can have the same function.

[0073] A comparison between homologous proteins or polypeptides reveals that the amino acid sequences of the ATP-binding domain and of the phosphorylated histidine catalytic domain are relatively conserved in general and are not prone to much variation. The amino acid sequence of the ATP-binding domain is preferably SEQ ID NO: 2, and the amino acid sequence of the phosphorylated histidine catalytic domain is preferably SEQ ID NO: 3.

[0074] The polyphenolic phytochemical phosphate synthetase (PPS) of interest in the present invention has 839 amino acids (SEQ ID NO: 13), a molecular weight of 94.9 kDa, a pI value of 4.81, a Mowse score of 765, a protein sequence coverage of 31%, and a unique peptide sequence

identified by protein mass spectrometry, with the gene of the target protein being a predicted protein gene (gene ID: 14103593) composed of 2520 bases. The nucleic acid sequence of the isolated, purified protein is known by way of gene cloning and DNA sequencing. More specifically, the nucleic acid sequences of those amino acid sequences capable of generating the substrate binding domain, the ATP binding domain, and the phosphorylated histidine catalytic domain are SEQ ID NO:4, SEQ ID NO: 5, and SEQ ID NO: 6 respectively.

**[0075]** The phosphorylation stated herein refers to the addition of a phosphate group to a protein or another type of molecule. This reaction plays an important role in energy metabolism and signal transduction in a living body and is a critical to biochemistry. Currently known phosphorylation entails kinase (which is a phosphotransferase) or phosphoprylase, both falling within the EC 2.7 category, and the reaction requires ATP as the source of energy and Mg<sup>2+</sup> ions as a cofactor. Generally, the aforesaid enzymes hydrolyze ATP and transfer γ-phosphate to the substrate. Protein kinase is the most common large-molecule phosphorylation enzyme, is responsible for modifying, through phosphorylation, a wide range of proteins with different functions, and is an essential means for regulating signal transduction in a living body. More and more physiological phenomena, such as whether an enzyme is activated or not, have been found to be related to the phosphorylation or dephosphorylation of protein. Certain amino acid sites on a protein molecule, such as the —OH functional group of serine, threonine, or tyrosine, or the imidazole ring of histidine, can be modified by protein kinase through phosphorylation such that the molecule is activated by the addition of phosphoric acid, and this phosphoric acid can be subsequently removed with protein phosphatase to render the molecule deactivated. There are also many examples in which similar reactions produce the opposite effects. As to small-molecule phosphorylation enzymes such as acetokinase, glycerokinase, arginine kinase, shikimate kinase, mevalonate kinase, and nucleoside kinase, they are responsible for such crucial catalytic reactions in the metabolic pathways in a living body as glycolysis, the biosynthesis of amino acids, the biosynthesis of cholesterol, and the biosynthesis of nucleotides. Phosphorylation enzymes can also be divided by the source of the phosphate group into the following two types. The first type uses a phosphoric acid monoester as the phosphate donor and is generally capable of hydrolyzing ATP and transferring γ-phosphate to the substrate. The second type uses a phosphonate diester or pyrophosphate as the phosphate donor instead.

**[0076]** Previous studies have found that *B. subtilis* BCRC 80517 can transfer the phosphate group to the hydroxyl group of a flavonoid, and based on its biochemical actions, this phosphorylation enzyme should be classified into the EC 2.7 category, in which, however, there are no enzymes for phosphorylating similar substrates. No research has so far been conducted on enzymes capable of phosphorylating polyphenolic phytochemicals, and there is even no published paper related to microbe-based production of polyphenolic phytochemical phosphates or enzymatically phosphorylated polyphenolic phytochemicals. This explains why the polyphenolic phytochemical phosphate synthetase obtained by purifying the bacterium body of *B. subtilis* BCRC 80517 in previous studies was classified into the EC 2.7.9 category after it was found by LC-MS/MS and a

bioinformatic analysis that the polyphenolic phytochemical phosphate synthetase; pyruvate, phosphate dikinase (PPDK); and phosphoenolpyruvate synthase (PEPS) have partially identical functional domains, similar sequences, and hence presumably the catalysis mechanism of PEP-utilizing enzymes. Enzymes such as PEP-utilizing enzymes carry out phosphate group transfer by forming a phosphohistidine intermediate and have three conserved domains: the PEP/pyruvate-binding domain, the PEP-utilizing domain/mobile domain, and the PEP-utilizing domain/C-terminal, in which the PEP-utilizing domain/mobile domain is a catalytic domain consisting of 15-30 amino acids, can rotate freely between the other domains, and includes a highly conserved histidine residue responsible for the transfer of the phosphate group. Such enzymes have the same working mechanism. The following paragraphs describe the reaction processes of certain PEP-utilizing enzymes, namely pyruvate, phosphate dikinase (PPDK); phosphoenolpyruvate synthase (PEPS); and rifampicin phosphotransferase (RPH).

**[0077]** PPDK (EC 2.7.9.1) can catalyze the reaction: ATP+pyruvate+Pi↔AMP+PEP+PPi, which is a reversible reaction. PPDK can be divided into three domains, which are the PPDK\_N, PEP-utilizers, and PEP-utilizers\_C domains. ATP (adenosine triphosphate) binds to the PPDK\_N domain at the N terminal to begin with and is thus hydrolyzed into AMP (adenosine monophosphate) and the pyrophosphate group (abbreviated as PPi). The PEP-utilizers domain then rotates to the N terminal such that PPi is transferred to the histidine of the PEP-utilizers domain, forming a phosphohistidine (His-PβPy) intermediate. Once the intermediate reacts with the phosphate group (Pi), the Py segment is cut off, forming His-Pβ and PPi. The PEP-utilizers domain then rotates to the C terminal to phosphorylate the pyruvate bound to the PEP-utilizers\_C domain, and PEP is formed as a result.

**[0078]** PEPS (EC 2.7.9.2) can catalyze the reversible reaction between ATP, pyruvate, and H<sub>2</sub>O to produce AMP, PEP, and Pi. The catalysis mechanism is the same as that of PPDK except that during the reaction process, the phosphohistidine (His-PβPy) intermediate reacts with H<sub>2</sub>O instead to cut off the Py segment and form His-Pβ and Py. PEPS and PPDK, therefore, have the same conserved domains. When a microbe is cultured with pyruvate or lactate serving as the carbon source, PEP production is the main reaction of PEPS. This indicates that PEPS has an important role in gluconeogenesis.

**[0079]** RPH (EC 2.7.9.6) is an enzyme capable of catalyzing phosphorylation-based modification of the OH group at C21 of rifampicin. It can catalyze the reaction between ATP, rifampicin, and H<sub>2</sub>O to produce AMP, rifampicinphosphate, and Pi. While the conserved domains of RPH are arranged in a different order from that of PPDK, the reaction mechanisms of RPH and PPDK are quite similar. The ATP-binding domain at the N terminal of the amino acid sequence of RPH functions as the PPDK\_N domain and is responsible for binding with ATP. The phosphorylated histidine catalytic domain at the C terminal of the amino acid sequence of RPH is the PEP-utilizing domain/mobile domain, includes a highly conserved histidine, and has the same reaction mechanism as its counterpart in PPDK and PEPS, except that its substrate-binding domain is located in

a middle portion of the sequence whereas the substrate-binding domain of PPDK is at the C terminal of the sequence.

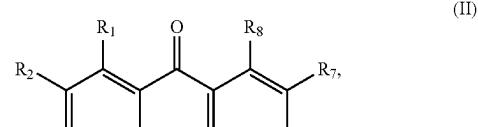
[0080] In a previous study, the amino acid sequence of the polyphenolic phytochemical phosphate synthetase obtained was compared with that of a known PPS, and the comparison result shows that the two synthetases have the same functional domains and similar primary sequences. Subsequent experiments further indicated that the two synthetases have the same enzyme mechanism. The polyphenolic phytochemical phosphate synthetase obtained in that study was named flavonoid phosphate synthetase (FPS) and was classified into the EC 2.7.9.x1 enzyme category. FPS can transfer two of the phosphate groups of ATP to a flavonoid and water.

**[0081]** In the present invention, the sequence alignment of amino acid sequence identity is obtained by any general amino acid sequence alignment method and amino acid sequence alignment tool. The amino acid alignment method is such as but not limited to: Needleman-Wunsch algorithm, Smith-Waterman algorithm, or Karlin & Altschul algorithm; and the amino acid sequence alignment tool is such as but not limited to: BLAST (Basic Local Alignment Search Tool) program, BLAT (BLAST-like Alignment Tool) program, Gapped BLAST program or FASTA program.

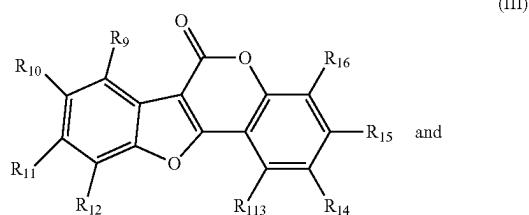
[0082] Regarding the catalytic sites stated herein, the inventor of the present invention has found a number of important catalytic sites through an extensive research, including Lys27, which is related to ATP binding, and His795, which is responsible for carrying and transferring the phosphate group. In some embodiments, the ATP-binding domain includes such catalytically active sites as Lys27, Arg102, and Glu282, all of which are related to ATP binding. In some other embodiments, the phosphorylated histidine catalytic domain includes His795, which is a catalytic site that carries and transfers the phosphate group. As for the important catalytic sites in the substrate-binding domain, the inventor has found that the catalytically active sites Asp627, His629, and His630 in the substrate-binding domain, as well as His795 (which is responsible for carrying and transferring the phosphate group) in the phosphorylated histidine catalytic domain, of the polypeptide in question are important active sites in the catalysis of phosphorylation. Under certain circumstances, the substrate-binding domain includes a conserved sequence whose amino acid sequence is DDHHFYIDAMILDAKAR (SEQ ID NO: 14).

**[0083]** The polypeptide of the present invention or micro-organisms containing nucleic acid sequences encoding the polypeptide can catalyze a substrate to their phosphate derivatives. Said substrate may be a polyphenolic phytochemical, for example: the substrate is a natural or modified curcuminoid, anthraquinones, chalcone, stilbenoid, coumestan or coumarin. More specifically, the curcuminoid is a natural or modified curcumin, bisdemethoxycurcumin, tetrahydrocurcumin or octahydrocurcumin; the anthraquinone is a natural or modified emodin, obtusifolin or aloe-emodin; the chalcone is a natural or modified phloretin, isoliquiritigenin or flavokawain A; the stilbenoid is a natural or modified resveratrol, pterostilbene, piceatannol or oxyresveratrol; the coumestan is a natural or modified coumestrol, wedelolactone or demethylwedelolactone; the coumarin is a natural or modified umbelliferone or 4-hydroxycoumarin.

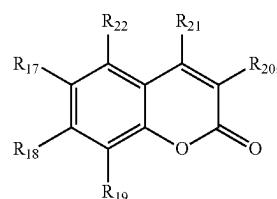
**[0084]** In terms of chemical structure, the substrate is selected from the group consisting of the following formulas:



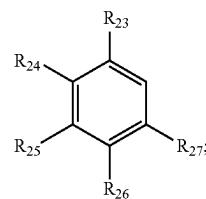
Ar1-L-Ar2(I),



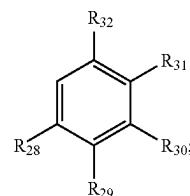
(IV)



[0085] wherein Ar1 is an aryl group of the following formula:



[0086] Ar<sub>2</sub> is an aryl group of the following formula:



**[0087]** L is a linking group comprising 3 to 7 backbone carbon atoms forming a chain linking Ar1 and Ar2 as the case may be, wherein L comprises at least one of a double bond, a carbonyl group and a hydroxyl group;

[0088] R1 to R8 are respectively H, (C1-C5)alkyl group, hydroxyl group, OR33, OCH2OR34, OCOR35, COR36, CO2R37, OCH2COOR38, OCH2 (OR39)2,

OC=ONHR40, halogen, nitro, amino, NR41R42, cyano group, mercapto group, SR43, S(O)qR44, (C1-C5)chloroalkyl group, (C1-C5)haloalkoxy group, (C2-C6)alkenyl group, (C2-C6)alkynyl group, (C3-C10) cycloalkyl group, (C6-C11)phenyl group or (C7-C12) benzyl group, wherein q is an integral of 1 to 3, and at least one of R1 to R8 is a hydroxyl group;

[0089] R9 to R16 are respectively H, (C1-C5)alkyl group, hydroxyl group, OR33, OCH2OR34, OCOR35, COR36, CO2R37, OCH2COOR38, OCH2 (OR39)2, OC=ONHR40, halogen, nitro, amino, NR41R42, cyano group, mercapto group, SR43, S(O)qR44, (C1-C5)chloroalkyl group, (C1-C5)haloalkoxy group, (C2-C6)alkenyl group, (C2-C6)alkynyl group, (C3-C10) cycloalkyl group, (C6-C11)phenyl group or (C7-C12) benzyl group, wherein q is an integral of 1 to 3, and at least one of R9 to R16 is a hydroxyl group;

[0090] R17 to R22 are respectively H, methoxy group or hydroxyl group, and at least one of R17 to R22 is a hydroxyl group, or R20 and R21, R17 and R18, R17 and R22, R18 and R19 or their combination are fused to form a (C3-C6)cycloalkyl group with hydroxyl group or a (C6-C10)aryl group with hydroxyl group;

[0091] R23 to R27 are respectively H, methoxy group or hydroxyl group, and at least one of R23 to R27 is a hydroxyl group;

[0092] R28 to R32 are respectively H, methoxy group or hydroxyl group, and at least one of R28 to R32 is a hydroxyl group;

[0093] R33 to R34 are respectively (C1-C5)alkyl group, (C1-C5)haloalkoxy group, (C2-C6)alkenyl group, (C2-C6)alkynyl group, (C6-C11)phenyl group or (C7-C12) benzyl group;

[0094] R35 is (C1-C5)alkyl group, (C1-C5)haloalkoxy group, (C6-C11)phenyl group or (C7-C12)benzyl group;

[0095] R36 is (C1-C5)alkyl group, (C1-C5)haloalkoxy group, (C2-C6)alkenyl group, (C2-C6)alkynyl group, (C6-C11)phenyl group or (C7-C12)benzyl group;

[0096] R37 to R40 are respectively (C1-C5)alkyl group or (C1-C5)haloalkoxy group;

[0097] R41 and R42 are respectively H, (C1-C5)alkyl group or (C1-C5)haloalkoxy group, one of which is H and the other is not H;

[0098] R43 and R44 are respectively H, (C1-C5)alkyl group or (C1-C5)haloalkoxy group.

[0099] The microorganism of the present invention comprises a nucleic acid sequence encoding the aforementioned polypeptide. The nucleic acid sequence is derived from *Bacillus* or *Streptococcus*. For example, the nucleic acid sequence is derived from *Bacillus subtilis*, *Bacillus halotolerans*, *Bacillus atrophaeus*, *Bacillus mojavensis*, *Bacillus velezensis* or *Bacillus amyloliquefaciens*. More preferably, it is derived from *Bacillus subtilis*. The polypeptides with the polyphenolic phytochemical phosphate synthetase activity of the above strains are homologous, and after comparison with the homologous amino acid sequences of other strains, the full-length amino acid sequence of the *Bacillus subtilis* (SEQ ID NO: 7) is 99% identical with SEQ ID NO: 13 of the present invention; the amino acid sequence of the *Bacillus halotolerans* (SEQ ID NO: 8) is 89% identical to SEQ ID NO: 13 of the present invention; the amino acid sequence of the *Bacillus mojavensis* (SEQ ID NO: 9) is 88% identical to the SEQ ID NO: 13 of the present invention; The

amino acid sequence of the *Bacillus atrophaeus* (SEQ ID NO: 10) is 75% identical to the SEQ ID NO: 13 of the present invention; the amino acid sequence of the *Bacillus velezensis* (SEQ ID NO: 11) is 71% identical to the SEQ ID: 13 of the present invention; the amino acid sequence of the *Bacillus amyloliquefaciens* (SEQ ID NO:12) is 70% identical to SEQ ID NO: 13 of the present invention.

[0100] The above-mentioned microorganism can normally express the nucleic acid sequence after transfer or transformation of the genetic material of the above-mentioned polypeptide (sequentially comprising the amino acid sequence of the ATP-binding domain, the substrate-binding domain and the phosphorylated histidine catalytic domain) through genetic engineering or molecular biotechnology.

[0101] The aforementioned microorganism may be a microorganism that has been genetically modified to express the aforementioned polypeptide, which may include genetic modification of the organism to enhance or enhance the production of the polypeptide in the organism. A genetically modified microorganism may be a genetically modified bacterium, unicellular organisms, microalgae, fungi, etc. This genetically modified microorganism has a genome modified (i.e. mutated or altered) from its normal form (i.e. wild-type or naturally occurring) such that the desired result can be achieved. Genetic modification of microorganisms can be accomplished using typical strain development and/or molecular genetic techniques. Such techniques are known in the art and are generally disclosed for use with microorganisms. A genetically modified microorganism may be a microorganism in which a nucleic acid molecule has been inserted, deleted, or modified (i.e. mutated; such as by nucleotide insertions, deletions, substitutions, and/or inversions) in such a way that this kind of modifications can provide the desired effect on the microorganism.

[0102] From another aspect, a method for synthesizing a polyphenolic phytochemicals phosphate derivative provided herein comprises: exposing a polyphenolic phytochemical to the aforementioned polypeptide or to a microorganism comprising a nucleic acid sequence encoding the aforementioned polypeptide for converting the polyphenol phytochemical to its phosphate derivatives.

[0103] The method of the present invention for synthesizing a polyphenolic phytochemical phosphate derivative can transform a polyphenolic phytochemical into its phosphate derivatives. Those polyphenolic phytochemical phosphate derivatives have a higher absorption rate and higher bioavailability than non-phosphorylated polyphenolic phytochemicals and, thanks to their advantageous bioactivity, can be used to make food, pharmaceuticals, industrial materials, and so on. Some examples of the aforesaid food are nutritional supplements, health food, functional food, baby food, and food for the elderly. Such food may be a solid, a fluid, a liquid, or a mixture of the above, preferably a liquid. When a pharmaceutical (e.g., a prodrug) is made, there is no special limitation on its dosage form. For example, the dosage form may be a solution, a paste, a gel, a solid, powder, or any other forms. If necessary, the pharmaceutical may include another pharmaceutically active ingredient (e.g., an anti-inflammation ingredient) or an auxiliary ingredient (e.g., a lubricating ingredient or a vehicle ingredient).

[0104] The following non-limiting embodiments of the invention are provided primarily to illustrate aspects of the invention and the benefits achieved. However, it should be understood that these examples are only for helping to make

the present invention easier to understand, and are not intended to limit the scope of the present invention.

#### Embodiment

[0105] *Bacillus subtilis* BCRC 80517 was obtained through a screening process and was deposited at the Bioresource Collection and Research Center of the Food Industry Research and Development Institute. *Bacillus amyloliquefaciens* BCRC 23350 and *Bacillus mojavensis* BCRC 17124 were purchased from the Bioresource Collection and Research Center. Any bacterial strain that required short-term storage was kept in a 4° C. solid-state nutrient agar (NA) culture medium, with continued subculturing to ensure the activity of the strain. Any bacterial strain that required long-term storage was kept at -80° C. in a 25% glycerol solution.

[0106] The gene of the polyphenolic phytochemical phosphate synthetase (PPS) for use in this embodiment was cloned separately from *B. subtilis* BCRC 80517, *B. amyloliquefaciens* BCRC 23350, and *B. mojavensis* BCRC 17124 such that the recombinant genes pps\_Bsub:pET47b (+), pps\_Bamy:pET47(+), and pps\_Bmoj:pET47b(+) were formed. *E. coli* DH5a was used as the cloning host, and *E. coli* BL21 (DE3) as the expression host. The bacterial strains were kept in -80° C. 25% glycerol solutions for long-term storage.

[0107] To start with, the bacterial strains were activated, and seed culturing was subsequently carried out. IPTG was then used to induce high-level expression of the recombinant protein genes. The steps performed for each strain are as follows. 100 mL of liquid TB culture medium containing 50 µg/mL kanamycin was inoculated with 5 mL of seed. After the seed was cultured at 37° C. and 150 rpm for 2 hours, the OD600 absorbance value reached somewhere between 0.6 and 0.8. Then, 40 mL of 500 mM IPTG was added to induce expression of the recombinant protein, with the final IPTG concentration being 0.5 mM. The culturing continued at 16° C. and 100 rpm for three days, before the bacterium body was centrifugally collected (6,000 rpm, 4° C., 20 min). The collected bacterium body was washed twice with an enzyme buffer solution, and the resulting bacterial pellets were collected and stored in a -20° C. refrigerator.

[0108] The bacterium body obtained from 100 mL of the bacterial solution was re-suspended with 30 mL of cell lysis buffer solution and then vibrated until there were no noticeable clumps in the solution. After that, the solution was subjected to two ultrasonic disruption sessions in an iced water bath. Each session lasted 10 minutes (which included repeated cycles each consisting of 9 seconds of disruption and 5 seconds of rest), and there was a 5-minute rest between the two sessions. Following the disruption, low-temperature centrifugation was performed (15,000 rpm, 4° C., 30 min). The resulting supernatant was collected and filtered through a 0.22 µm PVDF membrane to become a crude enzyme extract. The crude enzyme extract was purified by FPLC, or more specifically by affinity chromatography, and an enzyme liquid was obtained as a result.

[0109] 50 µL of enzyme reaction liquid (which contained a 1 mM reaction substrate (pre-dissolved in DMSO), a 50 mM Tris-HCl buffer solution, 100 mM NaCl, 10 mM ATP, and 10 mM MgCl<sub>2</sub>, and which had a pH value of 7.8) and 50 µL of the enzyme solution (at a concentration of 100 µg/mL) were evenly mixed and allowed to react at 40° C. for 10 minutes, at the end of which 900 µL of methanol was

added to terminate the reaction. After vibration-based mixing and centrifugation (13,000 rpm, 4° C., 5 minutes), the resulting supernatant was collected, diluted, and then analyzed by HPLC. The activity unit was defined as the amount of enzyme required to yield 1 nmol of product per unit time.

#### I. Determination of the Enzymatic Activity of the Recombinant PPSs

[0110] In this embodiment, the effects of using the PPSs obtained to catalyze the phosphorylation of a plurality of polyphenolic phytochemical substrates were further evaluated by allowing a 2 mM polyphenolic phytochemical substrate, 10 mM Mg<sup>2+</sup>/ATP, and 200 µL of a reaction buffer solution containing 1 mg/mL (10 µM) recombinant PPS to react for 2 hours. The activity unit was defined as the amount of enzyme required to yield 1 nmol of polyphenolic phytochemical phosphate derivative per unit time.

[0111] The catalyzing effects of the recombinant PPSs on each type of substrates are shown in FIG. 1, in which it can be seen that the recombinant PPSs were effective (indicated by the biotransformation rates) in phosphorylating various flavonoids, including genistein, and that the recombinant PPSs were also effective in phosphorylating such substrates as curcuminoids, anthraquinones, chalcones, stilbenes, coumestrols, and coumarins.

[0112] More detailed results are shown in FIG. 2 to FIG. 22.

[0113] With regard to curcuminoids, the phosphorylation results of curcumin (CUR), bisdemethoxycurcumin (BDMC), tetrahydrocurcumin (THC), and octahydrocurcumin (OHC) by the recombinant PPSs are shown in FIG. 2 to FIG. 5 respectively.

[0114] With regard to anthraquinones, the phosphorylation results of emodin (EMD), obtusifolin (OBF), and aloe-emodin (ALE) by the recombinant PPSs are shown in FIG. 6 to FIG. 8 respectively.

[0115] With regard to chalcones, the phosphorylation results of phloretin (PHL), isoliquiritigenin (ISL), and flavokawain A (FKA) by the recombinant PPSs are shown in FIG. 9 to FIG. 11 respectively.

[0116] With regard to stilbenes, the phosphorylation results of resveratrol (RES), resveratrol 3-O-phosphate (RES-3P), and resveratrol 4'-O-phosphate (RES-4'P) by the recombinant PPSs are shown in FIG. 12 to FIG. 14 respectively, and the phosphorylation results of pterostilbene (PTER), piceatannol (PCT), and oxyresveratrol (OXY) by the recombinant PPSs are shown in FIG. 15 to FIG. 17 respectively.

[0117] With regard to coumestrols, the phosphorylation results of coumestrol (CUM), wedelolactone (WDL), and demethylwedelolactone (DMWDL) by the recombinant PPSs are shown in FIG. 18 to FIG. 20 respectively.

[0118] With regard to coumarins, the phosphorylation results of umbelliferone (UMB) and 4-hydroxycoumarin (4HC) by the recombinant PPSs are shown in FIG. 21 and FIG. 22 respectively.

[0119] The experimental results show that the recombinant PPSs exhibited an unexpectedly wide promiscuity toward a diversity of species of polyphenolic phytochemicals. The PPSs succeeded in transforming different types of polyphenolic phytochemicals (namely flavonoids, curcuminoids, anthraquinones, chalcones, stilbenes, coumestrols, and coumarins) into their respective phosphate derivatives.

## II. Bioinformatic Analysis of the PPS Protein Family

**[0120]** Polyphenolic phytochemical phosphate synthetase (PPS) is referred to in the NCBI database as hypothetical protein BSNT\_10146 [*Bacillus subtilis* subsp. *natto* BEST195] (BAI87146), its coding gene being *yvkC* hypothetical protein [*Bacillus subtilis* subsp. *natto* BEST195] (gene ID: 14103593), which is composed of 2520 bases. PPS is a novel dinkinase (EC 2.7.9.X), whose amino acid sequence has been found, by an analysis based on the InterPro database, to have two conserved domains, namely Glu19-Glu310, which is an ATP-binding domain (IPR002192), and Ile734-Thr834, which is a phosphorylated histidine catalytic domain (IPR008279). From a comparison with the functional domains of pyruvate, phosphate dinkinase (PPDK) (EC 2.7.9.1) we further inferred that Lys311-His733 may be a substrate-binding domain, although no classification or research regarding this domain can so far be found in the InterPro database. In this embodiment, a search based on the primary sequence of PPS was conducted in the NCBI database in order to find a homologous protein family, and a bioinformatic analysis was performed to analyze the differences between the primary sequences of the PPS protein family.

### 1. Comparison of the Sequences of the PPS Protein Family

**[0121]** A comparison with the amino acid sequences of the PPSs obtained in this embodiment was conducted with the BLASTp program of the NCBI database to find the most similar 500 sequences, from which the similar amino acid sequences of *Bacillus subtilis*, *Bacillus halotolerans*, *Bacillus atrophaeus*, *Bacillus velezensis*, *Bacillus amyloliquefaciens*, *Bacillus pumilus*, *Bacillus safensis*, *Bacillus aryabhattai*, *Bacillus megaterium*, *Aneurinibacillus migulanus*, and *Paludifilum halophilum* were selected, based on a descending order of the degree of identity, for a multiple sequence comparison with the PPSs obtained in this embodiment. The multiple sequence comparison was carried out with ESPript v. 3.0, and the comparison results are shown in FIG. 23. According to the comparison results, both the ATP-binding domain from Glu19 to Glu310 at the N terminal and the phosphorylation catalytic domain from Ile734 to

**[0122]** Interestingly, the substrate-binding domain in the middle shows marked sequence variations, in particular in the amino acid segments Asp302-Gln322 and Lys378-Glu388. It can therefore be inferred from the sequence comparison results that the major differences between homologous protein sequences of different species result from the different sequences of their substrate-binding domains. A highly conserved amino acid segment, however, was found in Ile625-Leu652 of the substrate-binding domain, in particular DDHHFYIDAMLDKAR (SEQ ID NO: 14). The high conservedness of this sequence indicates that the sequence may be an active site for enzymatic catalysis or an amino acid sequence critical to maintaining the three-dimensional structure.

### 2. Phylogenetic Tree of the PPS Protein Family

**[0123]** Furthermore, a total of 25 sequences—including those of the PPSs obtained in this embodiment and a number of sequences of different degrees of identity selected from the 500 sequences found with the BLASTp program of the NCBI database through a comparison with the amino acid sequences of the PPSs obtained in this embodiment, plus the sequence of LmRPH (EC 2.7.9.6)—were compared against one another to create the phylogenetic tree shown in FIG. 24. The tree was constructed with MEGA 6. More specifically, ClustalW was used to compare the whole amino acid sequences, and then the neighbor-joining method was used to establish the evolution relationship. As shown in FIG. 24, the more similar to the sequence of the PPS of interest in the present invention a homologous protein sequence is, the closer the phylogenetic relationship between them. It is worth particular notice that the foregoing recombinant PPS genes were cloned from *B. subtilis* BCRC 80517 (the expressed protein being BsPPS, which is the PPS of interest in the invention), *B. amyloliquefaciens* BCRC 23350 (the expressed protein being BmojPPS), and *B. mojavensis* BCRC 17124 (the expressed protein being BamyPPS) respectively, and that the degrees of identity of the recombinant PPS genes cloned from the latter two bacteria to the recombinant gene cloned from the first bacterium are not lower than 70% (see FIG. 24 and Table 1). It can therefore be inferred that a polypeptide having an amino acid sequence whose degree of identity to the amino acid sequence of the PPS of interest in the invention is not lower than 70% has the enzymatic activity for catalyzing binding of the foregoing substrates.

TABLE 1

Homologous protein	Whole sequence		ATP-binding domain		Substrate-binding domain		Phosphorylated histidine catalytic domain	
	Degree of identity (%)	Degree of similarity (%)	Degree of identity (%)	Degree of similarity (%)	Degree of identity (%)	Degree of similarity (%)	Degree of identity (%)	Degree of similarity (%)
BsPPS	100	100	100	100	100	100	100	100
BmojPPS	87.4	90.6	89.4	93.4	84.8	88.2	93.8	96.3
BamyPPS	70.1	76.7	77.5	82.5	63.8	71.0	82.5	91.3

Thr834 at the C terminal are highly conserved, and His795 is an active site for protein phosphorylation and is responsible for catalyzing transfer of the phosphate group such that the phosphorylation catalytic domain can be defined as a phosphorylated histidine catalytic domain.

## III. Simulation of the PPS Protein Structure

**[0124]** The experiment described in this section aimed to predict a possible tertiary structure of the PPS of interest in the present invention by homology modeling, or more specifically by SWISS-MODEL, and to predict possible

important catalytic sites of the PPS of interest according to a comparison with the template structures.

#### 1. Search for a Template with which to Establish the Simulated Structure

**[0125]** An online analysis of the protein sequence of the PPS of interest in the present invention was carried out through SWISS-MODEL in order to search the SWISS-MODEL Repository for a known protein structure with a similar sequence that could serve as a template. Currently, the most similar known structure to the PPS of interest is rifampin phosphotransferase from *Listeria monocytogenes* (LmRPH), i.e., EC 2.7.9.6 (Qi et al., 2016; Peter J. Stogios et al., 2016), which has a degree of identity of about 26%, a coverage of 95%, and a total of six structures, each co-crystallized with a different ligand, as shown in its structure data in the PDB database. In the six structures, 5FBU, 5FBT, 5FBS, and 5HV1 are structures with a stable phosphorylated histidine catalytic domain and a stable substrate-binding domain, and 5HV2 and 5HV3 are structures with a stable ATP-binding domain and a stable substrate-binding domain. Each of the six structures was used as a template with which to predict the tertiary structure of the PPS of interest by homology modeling.

#### 2. Establishment and Analysis of the Simulated PPS Structure

**[0126]** Once the tertiary structures were constructed with the templates by homology modeling respectively, their Qualitative Model Energy ANalysis (QMEAN) values were calculated with software. The QMEAN value is a composite score for the distribution of the major geometrical structures of a simulated protein structure and has a range of reliability from 0 to -4. The closer to 0, the more reliable the simulation result. As we intended for the phosphorylated histidine catalytic domain to swing to the stable structure of the substrate-binding domain, thereby allowing the catalytically active domain around His795, which is known to be responsible for catalyzing transfer of the phosphate group, to be found, the template-based simulated structure with the lowest QMEAN value, i.e., the simulated PPS structure based on the template 5FBT (QMEAN=-4.06), was selected for use, and the selected structure was observed with PyMOL. The simulated PPS structure was then divided as follows with reference to the functional domains of the structure of the LmRPH template: 1Met-Thr300 was defined as the ATP-binding domain (ABD), 301Thr-His733 as the substrate-binding domain (SBD), and Ile734-Ser839 as the phosphorylated histidine catalytic domain (pHis-CD), as shown in FIG. 25. ABD and pHis-CD are highly conserved in the primary sequence, and their structures and catalysis mechanisms are known. To better study the structure of SBD, SBD was particularly extracted to facilitate observation of its structural details. After an observation and comparison of the secondary structures of LmRPH and of the simulated PPS structure, SBD was further divided into Arm1 (Thr301-Lys392, Ala701-His733), Apex (Glu393-Asn487, His580-Gln700), and Arm2 (Lys488-Tyr579) as shown in FIG. 26 and was viewed as having a cross shape, with Apex serving as the central axis, and Arm1 and Arm2 located on two lateral sides of Apex respectively and enclosing HD in an “embracing” manner such that a hydrophobic pocket is

formed therebetween. The hydrophobic pocket might be a catalytically active site of the PPS.

#### IV. Analysis of Important Catalytically Active Sites of the PPS of Interest

##### 1. Prediction of Important Catalytically Active Sites of the PPS of Interest

**[0127]** To begin with, we compared the primary sequences of the ATP-binding domains and phosphorylated histidine catalytic domains of the PPS of interest in the present invention and of LmRPH and found two important catalytic sites, namely Lys27, which is related to ATP binding, and His795, which is responsible for carrying and transferring the phosphate group. To find an important catalytic site in the substrate-binding domain, the amino acids around the His795 site (which is responsible for carrying the phosphate group to facilitate catalysis) of the simulated PPS structure were observed in greater detail and were compared with the major amino acids around His825 (which is known to have important functions) of the LmRPH template. The active rifampin-phosphorylation-catalyzing domain of LmRPH is such that after His825 carries the phosphate group from ATP to the substrate-binding domain, the transferred phosphate group is stabilized by the electrically charged or polar amino acids of Arg666, Glu667, Lys670, and Gln337. Moreover, the Apex of LmRPH has a highly conserved REXXK motif, which is an important catalytically active site. Interestingly, the Apex structure of the simulated PPS structure also has a highly conserved segment, or more specifically a continuous electrically charged KDDHH segment consisting of Lys626, Asp627, Asp628, His629, and His630. A structural observation reveals that this segment is also located around the phosphorylation site His795 and forms a tightly enclosed structure as shown in FIG. 27. It was also found that Met328, Glu329, Ser330, and Asn331 in Arm1 of the simulated PPS not only might correspond in position to Gln337 of LmRPH, but also are similar to Gln337 in being polar. In addition, we searched outward from this conjectural active domain in order to find more active sites that may be related to substrate binding, and a structural observation plus the comparison results in section II regarding the activity of different homologous protein sequences toward different substrates led to the following findings: Glu349, Tyr350, Lys378, Asn379, Gln383, and Gln384 in Arm1, which amino acids have significantly different sequences from one another as shown in FIG. 28, might be the amino acids in the PPS of interest that are related to substrate binding.

##### 2. Verification of the Predicted Important Catalytic Sites by Performing Site-Directed Mutagenesis on the PPS of Interest

###### 2-1 Creation of Site-Directed Mutant Strains of the PPS of Interest

**[0128]** To verify whether the amino acids predicted from the simulated PPS structure are important catalytic sites, site-directed mutagenesis was conducted by substituting an amino acid under discussion with an amino acid with a different property (e.g., by substituting the electrically charged Lys or the polar Asn with Val, which is neither electrically charged nor polar) and thereby changing the catalytic activity of the protein. Generally, an amino acid of interest is substituted with alanine (Ala), which is the

smallest residue, in order to change the charge state or polarity of the site of the amino acid and thus deprive the protein of its ability to bind with a substrate. We performed site-directed mutagenesis on the predicted catalytic sites stated in the previous paragraph by substituting those amino acids with Ala, and the following site-directed mutant strains of the PPS of interest in the present invention were created as a result: H795A, K27A, K626A, D627A, D628A, H629A, H630A, M328A, E329A, S330A, N331A, E282A, Y350A, K378A, N379A, R102A, R296A, Q294A, Q383A, and Q384A.

[0129] More specifically, the previously constructed PPS expression plasmid pps\_Bsub:pET47b(+) was subjected to a polymerase chain reaction (PCR) into which a mismatched primer set was introduced. After that, DpnI was used to catalyze decomposition of the plasmid template, which was methylated in the first place. The mutant plasmid mutant-pps\_Bsub:pET47b(+) was then cloned in *E. coli* DH5α by way of transformation, and the cloned mutant-pps\_Bsub:pET47b(+) was extracted with a plasmid DNA extraction kit and sent to Genomics Bioscience & Technology Co., Ltd. for sequencing. The mutant-pps\_Bsub:pET47b(+) whose sequence had been ascertained was then expressed in *E. coli* BL21 (DE3) by way of transformation.

#### 2-2 Expression of the PPS of Interest and its Protein Mutant Strains

[0130] The protein expression strain pps\_Bsub:pET47b(+)/BL21 (DE3) was cultured and added with IPTG to induce its expression. The resulting bacterium body was subjected to ultrasonic disruption to produce a crude enzyme extract. The crude enzyme extract underwent preliminary purification in a HisTrap™ HP column and was collected in a fraction collector, before the PPS expression was analyzed by SDS-PAGE. The analysis results are shown in FIG. 29. The crude enzyme extract had a total protein concentration of 10 mg/mL and was overexpressed beyond the bearing capacity of the column such that while the sample was being injected, a large amount of protein flowed out without being adsorbed. When the unadsorbed protein was washed with a buffer solution containing 40 mM imidazole, only a light-color band showed. When eluting in the end with a buffer solution containing 200 mM imidazole, however, a thick noticeable band was observed at 95 kDa for the second and the third columns, indicating the presence of the PPS. The eluents of the two columns were combined and subjected to desalting chromatography in a HiTrap desalting column, with the protein concentration eventually diluted to 1 mg/mL as analyzed by the Bradford method. The relative specific activity of the PPS protein mutant strains is plotted in FIG. 30.

#### 2-3 Determination of the Enzymatic Activity of the PPS of Interest and its Protein Mutant Strains (with Genistein Used as the Catalyst Substrate in this Embodiment)

[0131] Once an enzyme was prepared, its protein concentration was diluted to 0.1 mg/mL. The enzyme and the enzyme reaction liquid were mixed at a ratio of 1:1 (v/v) and were allowed to react at 40° C. for 10 minutes, at the end of which methanol was added to dilute the protein tenfold and thereby terminate the reaction. The changes in concentration of genistein (GEN) and G7P (a product of GEN phospho-

rylation) were analyzed. The catalytic activity of the PPS of interest in the present invention and its mutant strains were subsequently calculated and compared, as shown in FIG. 30. According to the calculation and comparison results, the K27A protein mutant strain lost its activity because its ability to bind with ATP was destroyed, H795A exhibited a total loss of PPS activity because of its incapability to carry and transfer the phosphate group, Lys27 and His795 were two important catalytic sites in the ATP-binding domain, and Arg102 and G282 were also important sites in the ATP-binding domain. As to the predicted catalytic sites in the substrate-binding domain, D627A, H629A, and H630A exhibited a significant decrease in protein activity; had little, if any, activity for catalyzing binding of genistein; and were important catalytic sites (i.e., D627, H629, and H630) in the active domain for stabilizing the phosphate group. The activity test results of other protein mutant strains show no total loss of activity but a trend of decrease in activity. In particular, E329A and K378A exhibited a relatively significant decrease in activity and might be amino acids that bound to and thereby stabilized genistein. However, while the aforesaid important catalytic sites in the active domain for stabilizing the phosphate group were inferred with relatively great certainty, the amino acids in Arg102 either had relatively poor prediction results or had important catalytic sites composed of non-polar amino acids (which when substituted with Ala produce little change in property) such that the combined use of site-directed mutagenesis and enzymatic activity determination lacked accuracy.

#### 2-4 Circular Dichroism Spectra of the PPS of Interest and its Protein Mutant Strains

[0132] Circular dichroism spectroscopy is a method commonly used to investigate protein structures. As the  $\alpha$ -helix,  $\beta$ -sheet, and random-coil conformations have different waveforms in a far-UV (with wavelengths ranging from 200 to 250 nm) circular dichroism spectrum, whether the secondary structure of a protein has changed can be determined according to whether the waveforms are changed.

[0133] While the importance of the aforesaid important catalytic sites to catalysis has been determined by site-directed mutagenesis and enzymatic activity determination, the change in enzymatic activity of a protein mutant strain is not necessarily the result of a change in its three-dimensional structure caused by amino acid substitution. A circular dichroism spectrum helps us observe the three-dimensional structure of a protein so that by measuring the far-UV circular dichroism spectra of the PPS of interest in the present invention and its protein mutant strains, it can be seen whether it was a change in the secondary structure of a PPS mutant strain that effected a change in its activity. Referring to FIG. 31, the PPS protein mutant strains have substantially identical waveforms in the far-UV spectra, and those waveforms resemble that of the  $\alpha$ -helix conformation, meaning the major secondary structure of the PPS protein mutant strains is a  $\beta$ -helix. This observation is consistent with the simulation results.

[0134] Conclusion: The homologous genes pps and homologous proteins PPS of the polyphenolic phytochemical phosphate synthetase (PPS) found in *Bacillus subtilis* BCRC 80517 in this embodiment exist extensively in various prokaryotes. Those homologous proteins have catalysis mechanisms partly similar to those of PPDK (EC 2.7.9.1) and LmRPH (EC 2.7.9.6), include highly homologous ATP-

binding domains and phosphorylated histidine catalytic domains, but differ greatly in their substrate-binding domain sequences.

[0135] It can be inferred from the enzymatic catalysis results of the PPSs derived respectively from *B. subtilis* BCRC 80517, *B. amyloliquefaciens* BCRC 23350, and *B. mojavensis* BCRC 17124 that a polypeptide whose amino acid sequence has a 70% or higher degree of identity to the amino acid sequence of the PPS of interest in the present invention has the enzymatic activity for catalyzing the biotransformation of a substrate.

[0136] The tertiary structure of the PPS of interest in the present invention was successfully simulated by homology modeling, or more specifically by SWISS-MODEL. The structures of the ATP-binding domain and of the phosphorylated histidine catalytic domain were predicted with relatively high accuracy because both domains are highly conserved. The substrate-binding domain has a cross-shaped structure, with Apex forming the central axis, and Arm1 and Arm2 located on two lateral sides of Apex respectively and enclosing the phosphorylated histidine catalytic domain to form a catalytically active domain. Possible important cata-

lytic sites of the PPS of interest were predicted from a comparison between the simulated structures and their templates.

[0137] PPS protein mutant strains were created by site-directed mutagenesis, which together with an analysis of the activity of enzymatic catalysis and circular dichroism spectroscopy (which ascertained the secondary structure) proved that Lys27 and His795 are important catalytic sites in the ATP-binding domain and in the phosphorylated histidine catalytic domain respectively, and that Asp627, His629, and His630 are important catalytic sites for stabilizing the phosphate group-binding domain at His795.

[0138] According to the above, the present invention has ascertained important catalytic sites of a polypeptide so that the polypeptide can be provided with a continuous electrically charged amino acid segment at a specific position while being separated or engineered, and that when the polypeptide or a microbe having the nucleic acid sequence coding for the polypeptide is used to synthesize a polyphenolic phytochemical phosphate, the success rate of polyphenolic phytochemical phosphorylation or the yield of the synthetic polyphenolic phytochemical phosphate derivative will be increased in comparison with that of the prior art.

## SEQUENCE LISTING

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Sequence total quantity: 30
SEQ ID NO: 1      moltype = AA  length = 449
FEATURE          Location/Qualifiers
source           1..449
mol_type = protein
organism = Bacillus subtilis

SEQUENCE: 1
SFMITDMDN DFWLNMESNI EGPVSPPLFSS FIVPALEYGL KKSMQKPIG VVVDEVKLYR 60
GHIYSKNQGG QQPPSEDCGK ELPFILSEHM YDIINHTYLP FYRTLDQLAQ TEHTAESALD 120
AFQKLKAFYL TAYEEHFNIIV FPQILLTNKL QAMYQDIQGE SENAHFYEML TGKMNKSLET 180
DRCLWLFSME VQENPNLLTI FENNKPEQLQ EKLEQTDEGR HFLKNVHEFL QEYGWRSVKS 240
HDLIEQIWIW NPYFALANIQ NYVRNGYHFD NEFQTKKEKR EKLYNEFLEN IEDPGLRTEF 300
DRYYQWTLNS ANIKDDHHFY IDAMLDAKAR IFLLKIGELL AENGVIQDRE DLWFLYDDEV 360
EQALLHPVSL QEKAEKRRQI FHEYELAQAP AYLGPTKEQ LKAAEETIVGA VIEDEKNTE 420
HIFGIAASSG IATGPVKIR DANEFSQFA 449

SEQ ID NO: 2      moltype = AA  length = 289
FEATURE          Location/Qualifiers
source           1..289
mol_type = protein
organism = Bacillus subtilis

SEQUENCE: 2
AGAKGMNLRI LTKHGLPVDP GFIQTNALA RFMEDNQLQE TSENVESGII SGTFSDDELKD 60
ELTSSFYKLLR ESYRSVAVRSS SASEDLEGA SFAGQYETYL NIKTEEEFLA KVKECWASFF 120
SGRVSSYKKK MNNQIAEPLM GIVVQGLIDS EMSGVIFSRN PVTHDDRELL ISASYGLGEA 180
VVSGSVTPDT FIVNKSSFEI QKEIGAKEIY MESAAEGIAE KETSEDMRSR FCLTD EQVIE 240
LAEITKKTED LYGYPVDIEF GIADHQIYLL QARPITTIDQ DKKAAE EK 289

SEQ ID NO: 3      moltype = AA  length = 70
FEATURE          Location/Qualifiers
source           1..70
mol_type = protein
organism = Bacillus subtilis

SEQUENCE: 3
PGDVLVCKMT TPLWTSLFQD AKAIITDTGG ILSHAAIIAR EYGIPAVLGT RTATERLRDG 60
DIITVDGSSG 70

SEQ ID NO: 4      moltype = DNA  length = 1347
FEATURE          Location/Qualifiers
source           1..1347
mol_type = other DNA
organism = Bacillus subtilis

SEQUENCE: 4
agcttcatga ttaccgacac tgatatgaat gatttctggc ttaacatgga gtctaatttt 60
gaagggtcccg tgagtccgtt attttcatcc ttccatcggtc cggcatgttg atatggcttg 120
agaagagaca tgcaaaaatg tccgatttgtt gtatgttgtt atgaagataa actttatcgc 180

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ggacatattt	atccaaaaaa	ccaagggtgga	cagcagcctc	cttctgaaga	ctgcggcaaa	240
gagctttccc	cgattttatc	ggagcatatg	tatgacatca	tcaatcacac	atacctccct	300
tttaccggaa	cactggaca	gtcgcacaa	actgagcata	ccgcagaag	cgcactggat	360
gctttcaaa	aactaaaggc	cttttatctc	acggctttag	aagagcactt	caatatcggt	420
ttcccgaaaa	tccttttaac	aaacaactg	caaggcgatgt	atcaggacat	tcaaggagag	480
tcggaaaaacg	ctcattttta	tgagatgtcg	acaggaaaaaa	tgaacaatc	actggaaacg	540
gaccgttgtc	tatggctatt	ttctatggaa	gttcaggaga	acccgaactt	tctgaccatt	600
tttggaaaaca	acaagcctga	acagctccag	gagaatttag	aacaaacaga	tgaggggaga	660
cacttctgaa	agaacgtcca	tgaatttctt	caagaatacg	gatggagatc	tgtttaaaagt	720
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aattatgtcc	tgatggcta	tcatttttgac	aatgaatttc	agaaaaacgaa	agaaaaaacga	840
gagaaatttat	acaatgaattt	cttggaaaaac	atagaagatc	ccggtttgcg	cacccgattt	900
gaccgcattt	atcaatggac	actgaactct	gcaaatataa	aagatgatca	ccacttttat	960
attgacgcata	tgctggatgc	caaggcgaga	atctttctgc	tgaatgatgg	tgaatttgcg	1020
gccccaaaaacg	gtgtcattca	agatcgtag	gaccttgggt	tttataatga	cgacgaatg	1080
gaacaagcgc	ttcttcaccc	tgtatccctg	caagaaaaag	ctgaaaaacg	cacagagatt	1140
tttcatgagt	atgagctggc	ccaagcccc	gcctacctcg	gcaccccgac	aaaagaacag	1200
ctcaaaagcag	ctgaagaaat	tgteggcgct	gtgatagagg	atgaaaaaaa	cacagagaat	1260
catatttttg	gcattgcggc	atcaagcggc	attgcgacag	gtccgggtgaa	aatcattcgg	1320
gacgc当地	aattttctca	attecgcg				1347

SEQ ID NO: 5	moltype = DNA length = 936					
FEATURE	Location/Qualifiers					
source	1..936					
	mol_type = other DNA					
	organism = <i>Bacillus subtilis</i>					
SEQUENCE: 5						
atgaagaaaa	gaggggtttc	aaatatgtat	tctgttttat	ttcgcggc	agaagagtcc	60
agccagctgg	cttggagcaaa	aggaatgaat	ttgatttaat	tgaccaaaaca	cggttcttct	120
gttccggacg	ggttttatatt	tcaaaacgat	gwgctcgac	gttttatgga	ggacaaacccag	180
cttcaagaga	cgtgtaaaaa	cgtggaaacg	ggggatcattt	cttgcgtat	ttcggatgg	240
ctgaaaatgt	agctgactag	ttcttttat	aagcttagag	aatcatatcg	atccgtagcc	300
gtgcgttctt	ctgtcttc	ggaagattta	gaaggcgct	cattcgcccc	tcaatatgaa	360
acctacttaa	atatcaaaac	agagaaagag	tttctggcta	aagtggaaaga	atgctggcc	420
tcattttttt	ctgggggggt	cagcgatct	aagaaaaaaa	tgaacaatca	aatccgagag	480
ccgttaatgg	gaatagctgt	tcaggggctg	atcgatctcg	aatatgtcgg	tgttattttc	540
agccgc当地	ctgttaccca	tgatgtatg	gagcttttaa	tcaagcgocag	ctacgggttg	600
gggtgaagctg	ttgtttcagg	aagtgttacc	ccagacacgt	tcattgtttaa	taaatctcg	660
tttgagatctt	agaaagaaat	aggtgtcggaa	gaaatctaca	tggagtctgc	ggcagaagga	720
attgctgaaa	agaaagacag	tgaagacatg	cgccggccgt	tttgccttac	agatgtacaa	780
gtgtttaat	ttggctgaaat	cacaaaaaaa	acccggaccc	tgtacggata	tcctgtcgat	840
atagaatttg	gaatttgcgt	tcatcaaata	taccttctgc	aagctcgccc	gattacaacc	900
attgtatgg	acaaaaaggc	ggcagaagaa	aaacgc			936

SEQ ID NO: 6	moltype = DNA length = 237					
FEATURE	Location/Qualifiers					
source	1..237					
	mol_type = other DNA					
	organism = <i>Bacillus subtilis</i>					
SEQUENCE: 6						
cctggggacg	tactcgtttgc	caagatgacc	acaccgtat	ggaccacgt	gtttcaagac	60
gccaaagcga	taatttacaga	caaggcgcc	attttgc	acgctcgat	tatttgcct	120
gaatacggca	ttccacccgt	tctcggcaca	cgcacggca	ccgaaact	gcaacggcgt	180
gacatcatca	ctgttgcgg	tagcagcgcc	aaaatcaca	ttgtcagccg	gtcctgaa	237

SEQ ID NO: 7	moltype = AA length = 831
FEATURE	Location/Qualifiers
source	1..831
	mol_type = protein
	organism = <i>Bacillus subtilis</i>

SEQUENCE: 7						
MYSVLFRQAE	ESSQLAGAKG	MNLIKLTKHG	LPVPDGFIQ	TNALARFMED	NQLQETSENV	60
EGGIISGTFS	DELKDELTSS	FYKLRESYRS	VAVRSSSASE	DLEGASFAGQ	YETYLNKITE	120
EEFLAKVKEC	WASFFSGRVS	SYKKMNNQI	AEPLMGIVVQ	GLIDSEMSGV	IFSRNPVTHD	180
DRELLISASY	LGGEAVVSGN	TPDFTFIVNK	SSFEIQKEIG	AKEIYMESAA	EGIAEKETSE	240
DMRSRFCLTD	EQVIELAEIT	KKTEDLYGYP	VDIEFGTADH	QIYLLOQARPI	TTIDQDKKAA	300
EEKRSFMITD	TDMNDFWLNM	ESNIEGPVSP	LFSSFIVPAL	EYGLKKSQMK	FPIGVVVDEV	360
KLYRGRHIYSK	NQGGQQPPSE	DCGKELFPIL	SEHYDIIINH	TYLPFYRTLD	QLAQTEHTAE	420
SALEAFQKLK	AFYLTAYEEH	FNIVFPQILL	TNKLQAMYQD	IQGESENAHF	YEMLTGKMNK	480
SLETDRCLWL	PSVEVQENPN	LLAIFENNKP	EQLQECKLEQT	DEGRHFLKVN	HEFLQEYGWR	540
SVKSHDLIEQ	IWVENPYFAL	ANIQNYVRNG	YHFDNEFQKT	KEKREKLYNS	FLESIEDPGL	600
RTEFDRYYWQ	TLNSANIKDD	HHFYIDAMLD	AKARIFLLKI	HELLAENGVI	QDREDLWFLY	660
DDEVEQALLH	PVSLQKEKAEK	RRQIFHEYEL	AQAPAYLGP	TKEQLKAEEE	IVGAVIEDEK	720
NTENHIFGIA	ASSGIATGPV	KIIRDANEFS	QFAPGDVLVC	KMTTPLWTS	FQDAKAITD	780
TGGILSHAAI	IAREYGIPAV	LGTRTATERL	RDGDIITVTDG	SSGKITVVSR	S	831

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SEQ ID NO: 8	moltype = AA length = 831
FEATURE	Location/Qualifiers
source	1..831
	mol_type = protein
	organism = <i>Bacillus halotolerans</i>
<b>SEQUENCE: 8</b>	
MYSVLFRQAE ESSQLAGAKG MNLIKLTKHG LPVPGFIIQ TNALARFMED NQLHDSSENI 60	
ENAIIAIGTFS DELKNELTSS FYELRESYRS VAVRSSSASE DLEGASFAQQ YETYLNKTE 120	
EEFLAKVKEC WASFFSGRVS RYKKKMNNQI AEPLMGVVQ GLINSEISGV IFSQNPVTHD 180	
DRELLISASY GLGEAVVSGS VTPDTYIVHK ASFEIQKEMG LKEIYMESAA EGIAEKETSE 240	
DMRSRFCLTD EQVIELAEIT KKTTEALYGYP VLDEFGIADH KLYLLQARPI TTIEQDKKAA 300	
EEERNFMITD EDMDDFWLNM ESNIEGPVSP LFSSFIVPAL EYGLKKSMQQ FPIGVIVDEV 360	
KVYRGHIYSK NGQQQQPPE DSAEELFPIL SERMYDIHHN TYLPFYRTLD QLAQTEHTPE 420	
SALDAFKKL AFYLTAYPEEH FNIVFPQILL TNKLQAMYQN IQGETENSHY YEMLTGVMNK 480	
SLETDRRLWQ FSVEVRENPN LTAIFEHTQP QQLQKTLLEQI DEGRRFQKQV NAFLQEYGWR 540	
SVKSHDLIEQ TWAENPYYAL THIQNYVRNG YHPDNEFKKT IEKREKLYNE FLQSIEDPVL 600	
QKEFERYQW TLNSSNIKDD HHFYIDAMLD AKARVFLLKV GELAKSGVI RDREDLWFLY 660	
DDEVENALLH PVSLQEKAEK RRQIFHEYEL AQAPAYLGTP TKAQLKAAEE IVGAVIEDEK 720	
NTENDIFGIA ASSGIATGPV KLIRDASEFS RFAPGDILVC KMTPWLTSF QDAKAITD 780	
TGGILSHAAI IAREYGIPAV LGTRAATERL RDGDIVTVVDG NSGKITVVS S 831	
SEQ ID NO: 9	moltype = AA length = 831
FEATURE	Location/Qualifiers
source	1..831
	mol_type = protein
	organism = <i>Bacillus mojavensis</i>
<b>SEQUENCE: 9</b>	
MYSVLFRQAE ESSQLAGAKG MNLIKLTKHG LPVPGFIIQ TNALARFMED NQLHDSSENI 60	
ENAIIAIGTFS DELKNELTSS FYELRESYRS VAVRSSSASE DLEGASFAQQ YETYLNKTE 120	
EEFLAKVKEC WASFFSGRVS RYKKKMNNQI AEPLMGVVQ GLINSEISGV IFSQNPVTHD 180	
DRELLISASY GLGEAVVSGS VTPDTYIVHK ASFEIQKEMG LKEIYMESAA EGIAEKETSE 240	
DMRSRFCLTD EQVIELAEIT KKTTEALYGYP VLDEFGIANTH KLYLLQARPI TTIEQDKKAA 300	
EEERNFMITD EDMDDFWLNM ESNIEGPVSP LFSSFIVPAL EYGLKKSMQQ FPIGVIVDEV 360	
KVYRGHIYSK NGQQQQPPE DSAEELFPIL AERMYDIHHN TYLPFYRTLD QLAQTEHTPE 420	
SALEAFKKL AFYLTAYPEEH FNIVFPQILL TNKLQAMYQN IQGEAENSHP YEMLTGVMNK 480	
SLETDRRLWQ FSVEVRENPN LTAIFEHTEP QQLQKTLLEQI DEGRCFQKQV NEFLQEYGWR 540	
SVKSHDLIEQ TWAENPYYAL THIQNYVRNG YHPDNEFKKT IEKREKLYNE FLQSIEDPVL 600	
QKEFERYQW TLNSSNIKDD HHFYIDAMLD AKARVFLLKV GELAKSGVI RDREDLWFLY 660	
DDEVENALLH PVSLQEKAEK RRQIFHEYEL AQAPAYLGTP TKAQLKAAEE IVGAVIEDEK 720	
NTENDIFGIA ASSGIATGPV KLIRDASEFS RFAPGDILVC KMTPWLTSF QDAKAITD 780	
TGGILSHAAI IAREYGIPAV LGTRAATERL RDGDIVTVVDG NSGKITVVS S 831	
SEQ ID NO: 10	moltype = AA length = 834
FEATURE	Location/Qualifiers
source	1..834
	mol_type = protein
	organism = <i>Bacillus atrophaeus</i>
<b>SEQUENCE: 10</b>	
MYSVLFNKAE ESSQLAGAKG MNLIKMTKNG LPVPGFIIQ TNALARFMED NQLHETKEDI 60	
ENNIINGIFS DELKNELTAS FYELRESYAS VAVRSSSASE DLEGASFAQQ YETYLNVKTE 120	
DEFLGKVKEC WASFFSARVG RYKEKMNNH GKPLMGVVQ GLIESEVSGV IFSRNPVTHD 180	
DGELLISASY GLGEAVVSGS VTPDTFIVNK DSFAIQKEMG LKEIYMVSQS EGIAERETNE 240	
EMRNRYCLN EHIREALAALT KKTTEALYGYP VLDEFGIAEN TLYLLQARPI TTIEQDKEAA 300	
EEERDFMITD EDMNDFWLNM ESNIEGPISP LFSSLIVPAM EHGLKKRSEQ FPIGVIEEV 360	
KQYRGHIYSK QKGDPTEAKA KAAEAAEEL PHLAEMYGI LNKTFLPFYE TLDELSAASH 420	
TPESALNAFK KLKFAYMEAY DEHFNIVFPQ LLLLNTKLETM YQQVQGDTEH SHFHEMLTGK 480	
MNKSLETDRH LWLLSNEVKK NAALKQVFET HQAEELQETL AQTSQDGLFL DVKNFNFLEY 540	
GWRSVKSHDL IEQIWAENPY YALSHIQNYV RNYGYHFDNEF NKTIEKRKQL YNEFLQQIED 600	
EAFRKEYDRY YQWMLNSVI RDDHHFYIDA MLDAKARIPL LRIGENLADS GVIDDKEDLW 660	
YLYDDEIENA LHLPVPLQAK TAKRNFKE YELVHAPSIL GSPTAEQLKA AEDIVGSVTE 720	
DEKNTEDHIY CVAASSGIVS GPVKVIRDN EFSRFSPGDV LVCKMTPLW TSLFQDAKAV 780	
ITDTGGILSH AAIIAREYGL PAVLGTRAAT DRLRDGDVVV VDGTSGKITI VSRA 834	
SEQ ID NO: 11	moltype = AA length = 833
FEATURE	Location/Qualifiers
source	1..833
	mol_type = protein
	organism = <i>Bacillus velezensis</i>
<b>SEQUENCE: 11</b>	
MYSVRFQKAE ESSRSAGAKG MNLIKLTKHG LPVPGGFIQ TNALTRFMED NGLQADQEQ 60	
ERAJMNNTFS KELREELTAS FYELRESYAS VAVRSSSASE DLEGASFAQQ YETYLNKTE 120	
EEFLGKVKEC WASFFSARVS GYKEKMNNDT AEPLMGVVQ GLINSEISGV IFSRNPVTHD 180	
DGELMISASY GLGEAVVSGS VTPDTFIVNK DTFQIEKEIG LKEMYIVSKD EGVTEKETS 240	
DMRSRFCLDD ESVKELAMLT IKTEALYGYP VLDEFGFAEN KLYLLQARPI TTIAQDKAA 300	
DEEREFINMTP RDQKDFWLN M EANIEGPVSP LFASLIVPAL EYGLKESTKA FPVMGIEIER 360	
VKLHQGHVF S RQHKTDDKLP AEQLEALFPV LADRMYDIQ ETFLPFYQKL DELAHTDHTP 420	

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ETALDAFRNL	QDFYLKGYEE	HFNIVLPQVA	LNMILES MYG	QVEKENT SLL	YEMLAGVMNK	480
SLETDRQLWL	LSGQVKDSPE	LRRVFTVSH	DELHQTLLOQ	NEGKRFL EQV	GEFLQEY GWR	540
SVKSHDLIEQ	TWAENPYFAL	ANIQNYVRNG	YDFDSEFHKT	IEKRKQLYAD	FMDRIEDDG	600
RETFDRYYQW	TLSSSVIKDD	HHFYIDAMLD	AKARLCLLKI	GELLQKQGV	DDREDMWYLY	660
SDEVEKALAS	PVPMQEKAEE	RKQLFQQYQL	LEAPAYLGTP	TSEQQLKTAEQ	ITGSITEDEK	720
NTEHQIYGLA	ASSGIASGPV	KVIRDASEFS	RFSTGDLVLC	KMTTPLWTS	FQDAKAVITD	780
TGGILSHAAI	IAREYGLPAV	LGTRAATDRL	NDGDIVTV	DNGKITIVKR	ASC	833
SEQ ID NO: 12		moltype = AA	length = 833			
FEATURE		Location/Qualifiers				
source		1..833				
		mol_type = protein				
		organism = Bacillus amyloliquefaciens				
SEQUENCE: 12						
MYSVRFQKAE	ESSRSAGAKG	MNLIKLT KNG	LPVPDGFIQ	TNALTRFMED	NGLQADQE	60
ERAIMMNGTFS	KELREELTAS	FYELRETYAS	VAVRSSSASE	DLEGASFAGQ	YETYLN	120
EEFLGKVKEC	WASFFSARVS	GYKEKMNNDT	AEPLMGVVQ	GLINSEVSGV	IFSRNPVTH	180
DGELMISASY	GLGEAIVSGS	VTPDTFIVNK	DTPQIEKEIG	LKEMYIVSKD	EGVTEKET	240
DMRNRFC LDD	ESVKELAMLT	IKTELYGYP	VDFLEFGFAE	N KLYLLQARP	I TTIVQDQKAA	300
DEEREFIMTP	RDQKDFWLN	EANIEGPVSP	LFASLIVPAL	YEGLKESTKA	FPVMGIEIER	360
VKLHQGRVFS	RQHKTDDKLP	A EQLEALFPV	LADRMYDIQ	ETFLPFYQKL	DELAHTDHTP	420
ETALDAFRNL	QDFYLKGYAE	HFNIVFPQVA	LNMILES MYG	QVEKENT SHL	YEMLAGVMNK	480
SLETDRQLWL	LSGQVKDNP	LRRVFTVSH	DELHQTLLOQ	NEGKRFL EQV	GEFLQEY GWR	540
SVKSHDLIEE	TWAENPYFAL	ANIQNYVRNG	YDFDSEFHKT	IEKRKQLYAD	FMDRIEDDG	600
RETFDRYYQW	TLSSSVIKDD	HHFYIDAMLD	AKARLCLLKI	GELLQKQGV	DDREDMWYLY	660
SDEVEKALAS	PVPMQEKAEE	RKQLFQQYQL	LEAPAYLGTP	TSEQQLKTAEQ	ITGSITEDEK	720
NTEHQIYGLA	ASSGIASGPV	KVIRDASEFS	RFSTGDLVLC	KMTTPLWTS	FQDAKAVITD	780
TGGILSHAAI	IAREYGLPAV	LGTRAATDRL	NDGDIVTV	DNGKITIVKR	ASC	833
SEQ ID NO: 13		moltype = AA	length = 839			
FEATURE		Location/Qualifiers				
source		1..839				
		mol_type = protein				
		organism = Bacillus subtilis				
SEQUENCE: 13						
MKKRGVSNMY	SVLFQRAEES	SQLAGAKGMN	LIKLT KHGLP	VPDGFIIQTN	ALARPMEDNQ	60
LQETSENVES	GIISGTF SDE	LKDELTS SFY	KLRESYRSVA	VRSSSASEDL	EGASFAGQYE	120
TYLNIKTEEE	FLAKVKECWA	SFFSGRVSSY	KKMMNNQIAE	PLMGIVVQGL	IDSEMSGVIF	180
SRNPVTHDDR	ELLISASYGL	GEAVVSGSVT	PDTFIVNKSS	FEI QKEIGAK	EIYMESAEG	240
IAEKETSEDM	RSRFCLTDEQ	VIELAEITKK	TEDLYGYPVD	IEFGIADHQI	YLLQARPITT	300
IDQDKKAAEE	KRSFMI TDTD	MNDFWLN	NIESGPVSP	SSFIVPALEY	GLKKSQM	360
IGVVVDEVK	YRGHIYSKNQ	GGQQPPS EDC	GKELFPILSE	HMYDIINHTY	LPFYRTLDQ	420
AQTEHTEAES	LDAFQKLKAF	YL TAYEEHF	IVFPQILLTN	KLQAMYQDIQ	GESENAHFYE	480
MLTGKMN KSL	ETDRCLWLFS	MEVQENPNLL	TIFENNPKPEQ	LQEKLEQTDE	GRHFLKNVHE	540
FQYEYGRVSRV	KSHDLIEQI	VENPYFALAN	I QNYVRNGYH	FDNEFQTK	KREKLYNEFL	600
ENIEDPGLRT	EFDRYYQWTL	NSANIKDDHH	FYIDAMLD	AKRIFLLKIGE	LLAENGVIQD	660
REDLWFLYDDE	VEQALLHPV	SLQEKAEKRR	QIFHEYELAQ	APAYLGTPK	EQLKAAE	720
GAVIEDEKNT	ENHIFGIAAS	SGIATGPVKI	IRDANEFSQ	APGDVLVCKM	TTPLWTS	780
DAKAIITDTG	GILSHAAIIA	REYGIPAVLG	TRTATERL	RD GIITVDG	SS GKITVVSRS	839
SEQ ID NO: 14		moltype = AA	length = 16			
FEATURE		Location/Qualifiers				
REGION		1..16				
source		note = conserved motif				
		1..16				
		mol_type = protein				
		organism = synthetic construct				
SEQUENCE: 14						
DDHHFYIDAM	LDAKAR					16
SEQ ID NO: 15		moltype = AA	length = 839			
FEATURE		Location/Qualifiers				
source		1..839				
		mol_type = protein				
		note = subsp. natto	BEST195			
		organism = Bacillus subtilis				
SEQUENCE: 15						
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LQETSENVES	GIISGTF SDE	LKDELTS SFY	KLRESYRSVA	VRSSSASEDL	EGASFAGQYE	120
TYLNIKTEEE	FLAKVKECWA	SFFSGRVSSY	KKMMNNQIAE	PLMGIVVQGL	IDSEMSGVIF	180
SRNPVTHDDR	ELLISASYGL	GEAVVSGSVT	PDTFIVNKSS	FEI QKEIGAK	EIYMESAEG	240
IAEKETSEDM	RSRFCLTDEQ	VIELAEITKK	TEDLYGYPVD	IEFGIADHQI	YLLQARPITT	300
IDQDKKAAEE	KRSFMI TDTD	MNDFWLN	NIESGPVSP	SSFIVPALEY	GLKKSQM	360
IGVVVDEVK	YRGHIYSKNQ	GGQQPPS EDC	GKELFPILSE	HMYDIINHTY	LPFYRTLDQ	420
AQTEHTEAES	LDAFQKLKAF	YL TAYEEHF	IVFPQILLTN	KLQAMYQDIQ	GESENAHFYE	480
MLTGKMN KSL	ETDRCLWLFS	MEVQENPNLL	TIFENNPKPEQ	LQEKLEQTDE	GRHFLKNVHE	540

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FLQEYGVRSV	KSHDLIEQIW	VENPYFALAN	IQNYVRNGYH	FDNEFQKTK	KREKLYNEFL	600
ENIEDPGLRT	FFDRYYQWTL	NSANIKDDHH	FYIDAMLDAA	ARIFLLKIGE	LLAENGVIQD	660
REDLWFLYDD	EVEQALLHPV	SLQKEAKRKR	QIFHEYELAQ	APAYLGPTK	EQLKAAEIV	720
GAVIEDEKNT	ENHIFGIAAS	SGIATGPVKI	IRDANEFSOF	APGDVLVCKM	TTPPLWTSFQ	780
DAKAIITDTG	GILSHAAIIA	REYGIPAVLG	TRTATERLRL	GDIITVDGSS	GKITVVSR	839

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SEQ ID NO: 16	moltype = AA	length = 831
FEATURE	Location/Qualifiers	
source	1..831	
	mol_type = protein	
	note = subsp. subtilis str. 168	
	organism = <i>Bacillus subtilis</i>	

SEQUENCE: 16						
MYSVLFHQAE	ESSSQLAGAKG	MNLIKLTKHG	LPVPDGFIQ	TNALARFMED	NQLOETSENV	60
ESGIISGTF	DELKEELTSS	FYKLRESYRS	VAVRSSSASE	DLEGASFAGQ	YETYLNKTE	120
EEFLAKVKEC	WGSFFSGRV	SYKKMNNQI	AEPLMGIVVQ	GLIDSEMSGV	IFSRNPVTHD	180
DRELLISASY	GLGEAVVSGS	VTPDTFIVNK	SSFEIQKEIG	AKEIYMESSAA	EGIAEKETSE	240
DMRSRFCLTD	EQVIELAEIT	KKTEDLYGYP	VDIEFGIADH	QVYLLQARPI	TTIIEQDKAA	300
EERKSFMMTD	TDMNDFWLN	ESNIEGPVSP	LFSFFIVPAM	YEGLKKSQM	FPIGVVVDEV	360
KLYRGRHIYSK	NQGGQQPPSE	DCGKELFPIL	SEHMYDIIINH	TYLPFYETLQ	QLAQTEHTAE	420
SALDAFKLKA	AFYLTAYEEH	FNIVFPQILL	TNLQAMYQD	IQGESENAHF	YEMLTGKMNK	480
SLETDRCLWL	FSMEVQENPN	LLTIFENNKP	EQLQEKLQEQT	DEGRHFLKNV	HEFLQEQYWR	540
SVKSHDLIEQ	IWENPYFAL	ANIQNYVRNG	YHFDNEFQK	KEKREALYNE	FLENIEDPGL	600
RTEFDQYYQW	TLNSANIKDD	HHFYIDAMLD	AKARIFLLKV	GEllaengvi	QDREDLWFLY	660
DDEVEQALLH	PVSLQKEAK	RRQIFHEYELA	AQAPAYLGPT	TKEQLKAAEE	IVGAVIEDEK	720
NTENHIFGIA	ASSGIATGPV	KIIRDANEFS	QFAPGDVLVC	KMTTPLWTSF	FQDAKAIITD	780
TGGILSHAAII	IAREYGIPAV	LGTRTATERL	RDGDIITVDG	SSGKITVVSR	S	831

SEQ ID NO: 17	moltype = AA	length = 830
FEATURE	Location/Qualifiers	
source	1..830	
	mol_type = protein	
	organism = <i>Bacillus tequilensis</i>	

SEQUENCE: 17						
MYSVIFHQAE	ESSRLAGAKG	MNLIKLTKHG	LPVPDGFIQ	TNALARFMEE	NQLHETSESI	60
ESGIIACTFS	DELKEELTDS	FYKLRESYRS	VAVRSSSASE	DLEGASFAGQ	YETYLNKTE	120
EEFLAKVKEC	WGSFFSGRV	TYKKMNNQI	TEPLMGIVVQ	GLIDSEMSGV	IFSRNPVTHD	180
DRELLISASY	GLGEAVVSGS	VTPDTFIVNK	SSFEIQKEIG	TKEIYMESSAA	EGIAEKETSE	240
DMRKRFCLTD	EQVIELAEIT	KKTEDLYGYP	VDIEFGIANH	QVYLLQARPI	TTIIEQDKAA	300
EERKSFMMTD	ADMKDFWINM	ESNIEGPVSP	LFSFFIVPAM	YEGLKRNMQK	FPLGAIAEEV	360
KLYRGRHIYSK	NQGGHQPD	CGKEIFPILS	SERMYDIIKHT	YLPFYETLQ	LAQTDHTAES	420
ALDAFRKLKA	FYLRAYDEHF	NIVFPQMLLT	NKLQAMYQH	IQGESETAHFY	EMLTGKMNK	480
LETDRCWLWY	SVEVRENPNL	LAIFENTEPE	QLOQEKLQEQT	EGRHFLKNH	EFLQDYGWRS	540
VKSHDLIEQI	WAENPYFALA	NIQNYVRNG	HFDNEFQK	KEKREALYHF	LENIEDPHVR	600
EQFDQYYQW	LNSANIMDD	HHFYIDAMLD	AKARIFLLKV	ELLVKHGVQ	DREDLWFLY	660
DEVENALLH	VSLQKEAK	RRQAFHEYELA	KAPAYLGPT	KEQLKIAEEI	IVGAVIEDEK	720
NTENHIFGIA	ASSGIATGPV	IIRDASEFS	FASGDILVLC	MTPPLWTSF	FQDAKAIITD	780
TGGILSHAAII	IAREYGIPAV	GTTRTATERL	HGDIVTVGDG	SSGKITVVSR	A	830

SEQ ID NO: 18	moltype = AA	length = 831
FEATURE	Location/Qualifiers	
source	1..831	
	mol_type = protein	
	organism = <i>Bacillus vallismortis</i>	

SEQUENCE: 18						
MYSVLFHQAE	ASSSQLAGAKG	MNLIKLTKHG	LPVPDGFIQ	TNALTRFMED	NHLHQTS	60
EKIIIACTFS	DELKHELTNS	FYKLRSYRS	VAVRSSSASE	DLEGASFAGQ	YETYLNKTE	120
EEFLAKVKEC	WGSFFSGRV	SYKKMNNQI	AEPLMGIVVQ	GLIDSDVSGV	IFSRNPVTHD	180
DRELLISASY	GLGEAVVSGS	VTPDTFIVNK	SSFEIQKEIG	AKEIYMESSAA	EGIABRATSK	240
DMRSRFCLTD	EQVIELAEIT	KKTEDLYGYP	VDIEFGIADH	HVYLLQARPI	TTIIEQDKAS	300
EERKSFMMTD	ADMNDFWINM	ESNIEGPVSP	LFSIIIVPAM	YEGLKKNMRK	FPIGVVVDEV	360
KLYRGRHIYSK	SQDGQQPQTE	DCGELFPIL	SERMYDIIKHT	YLPFYETLQ	LAQTDHTAE	420
SALDAFRKLKA	AFYLMAYDEH	FNIVFPQMLL	TNLQAMYQH	IQGESENAHF	YEMLTGKMNK	480
LETDRCWLWY	SVEVRENPNL	LLSIFKNTKP	EQLQEKLQEQT	DEGKQFLRN	HEFLQEQYWR	540
VKSHDLIEQI	WAENPYFAL	NIQNYVRNG	HFDNEFQK	KEKREALYNE	FLENIEDPNL	600
RKEFDQYYQW	TLNSANIMDD	HHFYIDAMLD	AKARIFLLKV	GEllaengvi	QDREDLWFLY	660
DDDVENALLH	PVSLQDKA	RRQAFHEYELA	KAPAYLGNP	TKEQLKIAEE	IVGAVIEDEK	720
NTENHIFGIA	ASSGIATGPV	KVIRDASEFS	RFASGNILVC	KMTTPLWTSF	FQDAKAIITD	780
TGGILSHAAII	IAREYGIPAV	LGTRTATERL	RDGDIITVDG	STGKITVVSR	A	831

SEQ ID NO: 19	moltype = AA	length = 831
FEATURE	Location/Qualifiers	
source	1..831	
	mol_type = protein	
	organism = <i>Bacillus halotolerans</i>	

SEQUENCE: 19

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MYSVLFQRQAE ESSQLAGAKG	MNL1KLTKHG LPVPDGFIQ TNALARFMED NQLHDSSENI	60
ENAIITAGAFS DELKNELTSS	FYELRESYRS VAVRSSSASE DLEGASFAGQ YETYLNKITE	120
EEFLAKVKEC WASFFSGRV	SYKKMNNQI AEPLMGVVQ GLINSEISGV IFSQNPTVHD	180
DRELLISASY GLGEAVVSGS	VTPDTYIVHK ASFEIQKEMG LKEIYMESSAA EGIAEKETSE	240
DMRSRFCLTD EQVIELAEIT	KKTEALYGYP VDLEFGIANH KLYLLQARPI TTIEQDKAA	300
EERERNFMITD EDMDDFWLNM	ESNIEGPVSP LFSSFIVPAL EYGLKKSQQ FPIGVIVDEV	360
KVYRGHYISK NQGGQQQPPE	DSAAEELFPIL AEMYDIIH TYLPFYRTLD QLTQTEHTPE	420
SALEAFKKL AFYLTAYEEH	FNIVFPQILL TNKLQAMYQON IQGEAENSHF YEMLTGVMNK	480
SLETDRGLWQ FSVEVRENPS	LTAIFEHTEP QQLQKKLEQI DEGRRLFQKI NEFLQEYGWR	540
SVKSHDLIEQ TWAENPYAL	THIQNYVRNG YHFDNEFKKT IKREKLYNQ FLQSIEDSAL	600
QKEFERYQW TLNSSNIKDD	HHFYIDAMLD AKARVFLLK GELLAESGVI RDREDLWFLY	660
DDEVENALLH PVSLQEKAEK	RRQIFHEYEL AQAPAYLGTP TKAQLKAAEE IVGAVIEDEK	720
NTENDIFGIA ASSGIATGPV	KLIRDASEFS RFAPGDLILVC KMTPPLWTSL FQDAKAITD	780
TGGILSHAAI IAREYGIPAV	LGTRAATERL RDGDIVTVVG NSGKITVVSR S	831

SEQ ID NO: 20            moltype = AA length = 831  
 FEATURE                Location/Qualifiers  
 source                1..831  
 mol\_type = protein  
 organism = *Bacillus mojavensis*

SEQUENCE: 20  
 MYSVLFQRQAE ESSQLAGAKG MN11KLTKHG LPVPDGFIQ TNALARFMED NQLHDSSENI 60  
 ENAIITAGIFS DELKNELTSS FYELRESYHS VAVRSSSASE DLEGASFAGQ YETYLNKITE 120  
 EEFLNKVKEC WASFFSGRV RYKKMNNQI AEPLMGVVQ GLINSDISGV IFSQNPTVHD 180  
 DRELLISASY GLGEAVVSGS VTPDTYIVHK GSFEIQKEMG LKEIYMESSAA EGIAEKETSE 240  
 DMRSRFCLTD EQVIELTELT KKTEALYGYP VDLEFGIANH KLYLLQARPI TTIEQDKAA 300  
 EERERNFMITD EDMDDFWLNM ESNIEGPVSP LFSSFIVPAL EYGLKKSQQ FPIGVIVDEV 360  
 KVYRGHYISK NQGGQQLPAE DSAAEELFPVL SERMYDIIH TYLPFYRTLD QLAQTEHTPE 420  
 SALDAFKKL SFYLTAYEEH FNIVFPQILL TNKLQAMYQON IQGETENSHP YEMLTGVMNK 480  
 SLETDRGLWQ FSVEVRENPN LTALFEHAOP EHLQERLEQT DEGRQFLQKA NEFLQEYGWR 540  
 SVKSHDLIEQ TWAENPYAL THIQNYVRNG YHFDNEFKKT IKREKLYNQ FFQSIEDPAL 600  
 QKEFERYQW TLNSSNIKDD HHFYIDAMLD AKARVFLLK GELLAESGVI QDREDLWFLY 660  
 DDEVENALLH PVSLQEKAEK RRQIFHEYEL AQAPAYLGTP TEAQLKAAEE IVGAVIEDEK 720  
 NTENDIFGVA ASSGIATGPV KVIRDASEFS QFTPGDILVC KMTPPLWTSL FQDAKAITD 780  
 TGGILSHAAI IAREYGIPAV LGTRAATERL RDGDIVTVVG NSGKITVVSR S 831

SEQ ID NO: 21            moltype = AA length = 834  
 FEATURE                Location/Qualifiers  
 source                1..834  
 mol\_type = protein  
 organism = *Bacillus atrophaeus*

SEQUENCE: 21  
 MYSVLFNKAE ESSQLAGAKG MN11KMTKNG LPVPDGFIQ TNALARFMED NQLHETKED 60  
 ENNIINGIFS DELKNELTAS FYELRESYAS VAVRSSSASE DLEGASFAGQ YETYLNVKTE 120  
 DEFLGVKEC WASFFSARVG RYKEKMNNH GKPLMGVVQ GLIESEVSGV IFSRNPTVHD 180  
 DGELLISASY GLGEAVVSGS VTPDTFIVNK DSFAIQKEMG LKELYMVSQS EGIAERETNE 240  
 EMRNRFCLND EHIREALALT KKTEALYGYP VDLEFGIAEN TLYLLQARPI TTIDQDKEAA 300  
 EERDFMITSK EDMNDFWLNM ESNIEGPISP LFSSLIIPAM EHGLKKRSEQ FPIGVIEEV 360  
 KQYRGHYISK QKGDPTEAA KAAEAAEELF PHLAEMYDI LNKTFLPFYE TLDELSASH 420  
 TPESALNAFK KLKAFYMEAY DEHFNIVFPQ LLLNTKLETM YQQVQGDTEN SHFHEMLTGK 480  
 MNKSLETDRH LWLLSNEVKK NAALKQVFET HQAEEELQETL AQTSDGKLFL DKVNNEFLREY 540  
 GWRSVKSHDL IEOQIWAENPV RNYGHDFNEF NKTIEKRKQL YNEFLQQIED 600  
 EAFRKEYDRY YQWMLNSSV RDDHHFYIDA MLDAKARIFL LRIGEILTDS GVIDDKEDW 660  
 YLYDDEIENA LLHPVSLQAK AAKRREIFKE YELVHAPSYL GSPTAEQLKA AEDIVGSVTE 720  
 DEKNTEDHIY GVAASSGIVS GPVKVIRDAN EFSRFSPGDV LVCKMTPLW TSLFQDAKAV 780  
 ITDTGGILSH AAIIAREYGL PAVLGTRAAT DRLRDGDIVT VDGTSGKITI VSRA 834

SEQ ID NO: 22            moltype = AA length = 833  
 FEATURE                Location/Qualifiers  
 source                1..833  
 mol\_type = protein  
 organism = *Bacillus amyloliquefaciens*

SEQUENCE: 22  
 MYSVRFQKAE ESSRSAGAKG MN11KLTKNG LPVPDGFIQ TNALTRFIED NRLOTEEAQM 60  
 ERAIMNGTFS KELRDELAAS FYELRESYAS VAVRSSSASE DLEGASFAGQ YETYLNKITE 120  
 EEFLGVKEC WASFFSARVS GYKEKMNNDT AEPLMGVVQ GLINSEISGV IFSRNPTVHD 180  
 DGELMISASY GLGEAVVSGS VTPDTFIVNK GTFQIDKEIG LKEIYIVSQE EGVTEKETS 240  
 DMNRFCCLDD ENIKELAMLT IKTEELYGYP VDLEFGFAEN KLYLLQARPI TTIVQDQKA 300  
 DEERDFIMTP RDQKDFWLNM ESNIEGPVSP LFASLIVPAL EYGLKESTKR FPVMGIEIER 360  
 VKLHQGRVFS RQHQTDDEPP AEQLEALFP IADRMYDIH ETFLPFYQKL DKLAHNTNHTP 420  
 ETALDAFRNL QDFYLGKGYEE HFNVIFPQVA LNMMLESMYQ QIEKENTSSL YEMLAGVMNK 480  
 SLETDRQLWL LSGQVKDSPE LRRRVFTVSPA DELHQTLQS NEGKRFLEQV GEFLQEYGWR 540  
 SVKSHDLIEE TWAENPYAL ANIQNYVRNG YDFDSEFHKT IKRKQLYAA FMEKIEDDGF 600  
 RETFDRYYQW TLSSSVIKDD HHFYIDAMLD AKARLCLLKI GELLEKQGVI DDREDMWLY 660  
 DDEVENAKLAS PVPMQEKAEE RKQLFQQYQL LEAPAYLGTP TPEQLQVAEQ ITGSITEDEK 720  
 NTEHHIYGLA ASSGIATGPV KVIRDASEFS RFSSGDVLVC KMTPPLWTSL FQDAKAVITD 780

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TGGILSHAAI IAREYGLPAV LGTRAATDRL NDGDIVSVDG TNGKITIVKR ASC	833
SEQ ID NO: 23	moltype = AA length = 833
FEATURE	Location/Qualifiers
source	1..833
	mol_type = protein
	organism = <i>Bacillus velezensis</i>
SEQUENCE: 23	
MYSVRFQKAE ESSRSAGAKG MNLIKLTNG LPVPGGFIQ TNALTRFMED NGLQADQEQM	60
ERAIIINGTFS KELRDELIAS FYELRESYAS VAVRSSSASE DLEGASFAGQ YETYLNKTE	120
EEFLGKVKEC WASFFSARVS GYKEKMNDT AEPLMGVVQ GLINSEVSGV IFSRNPVTHD	180
DGELMISASY GLGEAIVSGS VTPDTFIVNK DTFQIEKBIG LKEMYIVSKL EGVTKEKTS	240
DMRNRFCLDD ESVKELAMLT IKTEELYGP VDLEFGFAEN KLYLLQARPI TTIAQDQKAA	300
DEEREFIMTP RDQKDFWLN M EANIEGPVSP LFASLIVPAL EYGLKESTKA FPVMGIEIER	360
VKLHQGHVFS RQHKTDDKL P AEQLEALFPV LADRMYDIQ KTFLPFYQKL DELAHTDHTP	420
ETALDAFRNL QDFYLKGYEE HFNIIVFPQVA LNMILESMYG QVEKNTSSL YEMLAGVMNK	480
SLETDRQLWL LSGQVKDSPE LRRRVFTVSHA DELHQTLQGS NEGRKRFLEQV GEFLQEYGR	540
SVKSHDLIEQ TWAENPYFAL ANIQNYVRNG YDPDSEFHKT IEKRKQLYAD FMDRIEDDG	600
RETFDRYYQW TLSSSVKDD HHFYIDAMLQ AKARLCLLKI GELLQKQGV DDREDMWYLY	660
SDEVEKALAS PVPMQEKAEE RKQLFQQYQL LEAPAYLGTP TSEQLKTAEQ ITGSITEDEK	720
NTEHQIYGLA ASSGIASGPV KVIRDASEFS RFSTGDVLC KMTPPLWTSL FQDAKAVITD	780
TGGILSHAAI IAREYGLPAV LGTRAATDRL NDGDIVSVDG TNGKITIVKR ASC	833
SEQ ID NO: 24	moltype = AA length = 833
FEATURE	Location/Qualifiers
source	1..833
	mol_type = protein
	organism = <i>Bacillus siamensis</i>
SEQUENCE: 24	
MYSVRFQKAE ESSRSAGAKG MNLIKLTNG LPVPDGFIQ TNALTRFMED NRLQADEEEM	60
ERAIIINGTFS KELRDELIAS FYELRESYAS VAVRSSSASE DLEGASFAGQ YETYLNKTE	120
EEFLGKVKEC WASFFSARVS GYKEKMNDT AEPLMGVVQ GLINSEVSGV IFSRNPVTHD	180
DGELMISASY GLGEAIVSGS VTPDTFIVNK DTFRIDKEIG LKEMYIVSKL EGVTKEKTS	240
DMRNRFCLDD ESIEKELAMLT IKTEELYGP VDLEFGFAEN KLYLLQARPI TTIAQDQKAA	300
DEEREFIMTP RDQKDFWLN M ESNIEGPISP LFASLIVPAL EYGLKESTKA FPVMGIEIER	360
VKLHQGHVFS RQHKTDDNLP PEQLEALFPV LADRMYDIQ KTFLPFYQKL DGLAHKNHTP	420
ETALEAFRNL QDFYLKGYEE HFNIIVFPQIA LNMTLESMYG QVEKDNASLL YEMLAGVMNK	480
SLETDRQLWL LSGQVKDNPE LLRVRFTASQA DELHQTLQGS DEGRKRFLEQV GEFLQEYGR	540
SVKSHDLIEQ TWAENPYFAL ANIQNYVRNG YDPDSEFHKT IEKRKQLYAA FMNRIEDDG	600
RETFDRYYQW ALSASVIIID HHFYIDAMLQ AKARLCLLKI GELLQKQGV DDREDMWYLY	660
SDEVEKALVT PVPMKEKTAE RKQLFQQYQL LEAPAYLGTP TPEQLKAAEQ VTGAITEDEK	720
NTEBHHIYGLA ASSGIASGPV KVIRDASDFS RFSSGDVLC KMTPPLWTSL FQDAKAVVTD	780
TGGILSHAAI IAREYGLPAV LGTRVATDRL NDGDIVSVDG TNGQITIVKR ASC	833
SEQ ID NO: 25	moltype = AA length = 843
FEATURE	Location/Qualifiers
source	1..843
	mol_type = protein
	organism = <i>Bacillus xiaoxiensis</i>
SEQUENCE: 25	
MYSVLFHAAE ESASLAGAKG VNLIKLNHG LPVPDGFIQ TNSFASFLSY HHLHPTEQML	60
EQKIKKNASFP SQMETELLSS FQSLLKTYPS VA VRSSSVAE DLEGASFAGQ YETYLNKTN	120
EEFLQAVKEC WSSYFAARVT EYKEEMVETE EDAMPLMAIVQ QGLIHSDVSG VIFSENPVSG	180
KTNEVMLTAS YGLGEAIVSG LVTPDTFIVD KETDSIEKSL GTKDLQIVPC QEGVIEQPVS	240
KERAGQFCFLH DDQLLQITK T KQVEALYGH GVDIEFGISN GTFYLLQARP ITTVLPKTAN	300
EVAGASFQIQ PEELQDFWIS MDDHMPGPTS PLFSSLIIPA LKSGMKSGE KYQVPDLNIK	360
DIKLYRGHLY SSPSMSPEAGT DTEPVDFESL FELFPRLSER MYHILEQNLF PLYEKLDROI	420
NQPLTTDEAI IGLKELKDIY LKAYDDHFDI VIPQVILSAM IEDMLVYTGT DQTQVLLHE	480
MMIGVMNKSLL ETDQKLQSYLA KEVLQDEELL QAPTNHATNP ERLYALNHTD KGKNFISKIE	540
EFLQIYWGWS VKSHDFTDET WVNEPDVFVLD IIRNNIQQYQS DFDLDEFAQAV LKRRETYEQF	600
MSQVKDFAEK TFKFEKLYHFA LQAANIRDDH HFYIDALMDA KARLYLLKIG ELLVQKGAI	660
HQEDLWYLYD DEVHKALTTS ITYDSVIQQR KIEMKENEAQ QPPAYTGSPS EAELQQVERM	720
LGSLRENNENN TNDVIHIGA SSGIVSGRK VITCADEFSQ FQKDDILVCK TTTPLWTSLF	780
RDAKAVITDS GGILSHSAII AREYMMPAVL GTRIATEKLK SGDLVTVGGA NGRIQLLKQH	840
SRA	843
SEQ ID NO: 26	moltype = AA length = 843
FEATURE	Location/Qualifiers
source	1..843
	mol_type = protein
	organism = <i>Bacillus pumilus</i>
SEQUENCE: 26	
MYSVLFHAAE ESAALAGAKG LNLIKLNHG LPVPDGFIQ TNSFSSFLSY HNLHQTEQNL	60
ADKIKKASFP SQMEAELLSS FQSLRKTYPS VA VRSSSVAE DLEGASFAGQ YETYLNKTN	120
EEFLQAVKEC WSSYFEARVT EYKEEMGENE EEMPLMAVVQ QGLIHSDVSG VIFSENPVG	180
KTNEMMLTAS YGLGEAIVSG IVTPDTFIVD KETLSIEKSL GTKELQIVPY QEGVIEQSVT	240

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EEMAGQFCLN DDQLVEITQI	TKQVETLYGH SVDIEFGIAN GTFYLLQARP ITAALSKAAD	300
ETAGASFQMQ PDELODFWIS	MDDHMPGPTS PLFSSLIIPA LKSGMKKNGE KYQVPDLNIK	360
DIKLYRGHLY SSPSLPFEASA	ETAPVFDESI FELFPHLSER MYEILEKNFF PFYEKLDRQM	420
KEPMTIEDAI VGFEKLKTFY	IQAYDDHFDI VIPQVILSTM IEDMLVTYTG DQSQVILLHE	480
MMIGVMNKS L TTDKKLSDFA	KSVLQDTELH QAFMKHEKNP ELLDALTOSE KGRHVISLE	540
EFLQVYGRWS VKSHDLTEET	WAENPEFILED IIRNNIQHQC DFDEEFQAQAV IKRQETYEHF	600
MSQVKDEAFK TKFETLYQFA	LQAANIRDHH HFYIDAMLD A KARVYLLKIG ELLVQKGAI P	660
HPEDLWYLY EEVHTALTTS	TSFDTVIAQR KIDMKDNEAI OPPAYMGPT EAELQQVERM	720
LGSLRENNEN TSDMIYGIGA	SSGIVSGRVK VITCAEEFSQ FRKDDILVCK TTPLWTSLF	780
RDAKAVITDA GGILSHSII AREYMPAVL GTRIATELQ SGDLVTVGDGS NGRIQILKQH	840	
ARV		843

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SEQ ID NO: 27      moltype = AA length = 843
FEATURE          Location/Qualifiers
source           1..843
mol_type = protein
organism = Bacillus safensis
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SEQUENCE: 27

MYSVLFHAAE ESASLAGAKG	LNLIKLNNHG LPVPDGFIKI TNSFASFLSY HNLQPAEQNL	60
SQKIKEASFP LQMEAELLSS	FQSLRKTYPS VAVRSSVAE DLEGASFAGQ YETYLNKTN	120
EEFLQAVKEC WSSYFAARVT	EYKEEMNENE EEMPLMAVVQ QGLIHSVSG VIFSENPVSG	180
KTNEEMMLTAS YGLGEAIVSG	LVTPTDFIVN KENFSIEKTL GAKELQISPQ QEGVIEEPVS	240
EEMAGQFCLN NDQLKEITQI	TKQVETLYGH GVDIEFGIAD GSFYLLQARP ITTALPKAVN	300
ETAGASFQMK PEELQDFWIS	MDDHMPGPTS PLFSSLIIPA LKSGMKKNGE KYQVPDMNIK	360
DIKLYRGHLY SAPDLPEAQE	GAPVFDESI FELFPHLSER MYEILEKNFF PFYEKLDRQM	420
NEPMTIKAEI IGFEELKDFY	IQAYDDHFDI VIPQVILSAI IEDMLVTYTG DQTQVLLHE	480
MMIGVMNKS L TTDKVLSDIA	KDVLQDPELH QAPIHHEKNS ELLYALKHSE KGKHFISKLE	540
DFLQVYGRWS VKSHDLTDET	WAENPKFID IIRNNIHCHC DFDEEFAKAV IKRQETYKHF	600
MSQVKDEAFK TKFETLYQFA	LQAANIRDHH HFYIDAMLD A KARVYLLKIG ELLAKKGAI P	660
HQEDLWLYD EEVQKALTTS	ISFDSVIQQR KIEMKENEDI OPPAYIGPT EAELQQVERM	720
LGSLRENNEN TSDMIHGIGA	SSGIVSGRVK VITCAEEFSQ FRKDDILVCK TTPLWTSLF	780
RDAKAVITDA GGILSHSII AREYMPAVL GTRIATELQ SGDLVTVGDGS NGRIQILKQH	840	
SRA		843

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SEQ ID NO: 28      moltype = AA length = 843
FEATURE          Location/Qualifiers
source           1..843
mol_type = protein
organism = Bacillus stratosphericus
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SEQUENCE: 28

MYSVLFHAAE ESASLAGAKG	LNLIKLNNHG LPVPDGFIKI TNSFASFLSY HNLQPAEQNL	60
SQKIKEASFP LQMEAELLSS	FQSLRKTYPS VAVRSSVAE DLEGASFAGQ YETYLNKTN	120
EEFLQAVKEC WSSYFAARVT	EYKEEMNENE EEMPLMAVVQ QGLIHSVSG VIFSENPVSG	180
KTNEEMMLTAS YGLGEAIVSG	LVTPTFIVD KENFSIEKTL GAKELQIVPF QEGVIEEPVS	240
EEMAGQFCLN DDQLKEITQI	TKQVETLYGH GVDIEFGIAD GSFYLLQARP ITTALPKAVN	300
ETAGASFQMK PEELQDFWIS	MDDHMPGPTS PLFSSLIIPA LKSGMKKNGE KYQVPDMNIK	360
DIKLYRGHLY SAPDLPEAQE	GAPVFDESI FELFPHLSER MYEILEKNFF PFYEKLDRQM	420
KEPMTIKAEI IGFEELKDFY	IQAYDDHFDI VIPQVILSAI IEDMLVTYTG DQTQVLLHE	480
MMIGVMNKS L TTDKVLSDIA	KDVLQDPELH QAPIHHEKNS ELLHALNHSE KGKHFISKLE	540
DFLQVYGRWS VKSHDLTDET	WAENPKFID IIRNNIQSOC DPDEEFAKAV IKRQETYKHF	600
MSQVKDEAFK TKFETLYQFA	LQAANIRDHH HFYIDAMLD A KARVYLLKIG ELLVQKGSI P	660
HQEDLWLYD EEVQKALTTS	ISFDSVIQQR KIEMKENEDI OPPAYIGPT EAELQQVERM	720
LGSLRENNEN THDVIHGIGA	SSGIVSGRVK VITCAEEFSQ FRKDDILVCK TTPLWTSLF	780
RDAKAVITDA GGILSHSII AREYMPAVL GTRIATELQ SGDLVTVGDGS NGRIQILKQY	840	
SRA		843

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SEQ ID NO: 29      moltype = AA length = 837
FEATURE          Location/Qualifiers
source           1..837
mol_type = protein
organism = Bacillus megaterium
```

SEQUENCE: 29

MYSLSFQKVD EVNEKVGAKA	LNLIKMKKEN LPIPDGFVIQ TEALKRYIEW NGINRQTDNI	60
HERILQGEIP IEIESDLIEA	FRNLKSSYTS VAVRSSSSAE DLEGASFAGQ YETYLNVKSP	120
EDFLSKVKAC WASFFTERVE	QYTQNMADF DEISMAVVQ GLIESEVSGV IFSQNPVTHN	180
TKEMMINASY GLGEAIVSGI	VTPDVYLVNK QTFEIEKEKG LKEVKIPLA EGVEEITTTE	240
DEQQRFCLT D EKIIELSEIT	KEVEALYQHP VDIEFGIQN QVYLLQARAI TTLTESKELT	300
PFKDISLSF EDMEEFWILN	DTSFSHAVSP LYASFIIIPAF SEGTAAASFKK LQFMPNRHL	360
KLYKGHIYTK TEPYKGDSNK	RFQANKELME SIYPILTKRM NQMIKEQFLP YYNKLDSTFE	420
GNLNLRQGKE ILQSLTDYK	TAYDLHFDIV MPQMSLNTKV EEEYKNLTNK KSGHDVYELL	480
TGEMMNKSLET DQQLSRLALT	VKGDAELTKI FEEECTETLL KKLEENKA AK SFMAEVDTFL	540

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KQYGYRSVVS HDFVGETWLE NPLHALLIIQ GYVNDGYHFD ENFKQTVKRR EQNYNEFLEQ	600
IADSTHKEEF KKYYQWALDA SVIRDDHHFY IDAMLDKAR LFLIKLGDLL VHHHVFLMKE	660
DIFFLYLDEV KSLLENPVDV TELVEKRKKE HAEHEQMPNL PRYFGVPEPA HLKEAEKYMG	720
AIEENDDNSE HSIKGASSS GTYTGKVVI SNTKEFYKLE KGDVLVCKTT TPLWTTLFQT	780
AGAVITDAGG ILSHSAIAR EYEIPAVVGT KISTNKLKD DIVMVDTNG IVTLLNE	837
SEQ ID NO: 30	moltype = AA length = 837
FEATURE	Location/Qualifiers
source	1..837
	mol_type = protein
	organism = Bacillus aryabhattai
SEQUENCE: 30	
MYSLSFQKVD EANERVGAKA LNLIKMKKEN LPIPDGFVIQ TEALKRYIEW NGIDRHTDNI	60
HERILHGEIP MEIETDLIEA FQTLKSSSAE VAVRSSSSA DLEGASFAQQ YETYLNVKSI	120
EDFLSKVKICP WASFFTERVE QYTQNMYADF DKISMAVVQ GLIESEVSGV IFSQNPVTHN	180
TKEMMINASY GLGEAIVSGL VTPDVYLINK QTPEIEKEKG LKEVKIPLD EGVEEETTE	240
DEQOKFCLTN EKIIIELAEIT KEVEALYQHP VDIEFGIQYD QVYQLQARAI TTLAEDNKL	300
SFOKDISL SFDMEEFWILN DTSFSHAVSP LYASFIIIPAF SEGTAAASFQK LNFIFPNRLN	360
KVYKGHIYTR TEPFKGDSK RSQKHKEML SIYPIILTKRM NQIIKEQFLS YYDKLDSFTR	420
ENPNLQRGKE ILQNLADFYK TAYDLHFDIV IPQMSLNNTTV EESYKNLTNK KSGHDVYELL	480
TGKMNKSLET DQQLSRLALT VKRDSELTEI FQEECTETLL KKLEGNKA AK SFMAEVDTFL	540
KQYGYRNVVS HDFVGETWLE NPLHALSIIQ GYVNDGYHFD ENFKQTVKRR EQNYNEFLEQ	600
IADSKHKEEF KKYYQWALDA SVIRDDHHFY IDAMLDKAR LFLIKLGDLL VHHHVFLMKE	660
DIFFLYLDEL ESLLENPVDV TELVEKRKKE HAEHEQMPNL PRYFGVPEPA HLKEAEKYMG	720
AIEENDDNSE HSIKGASSS GTYTGKVVI SNTKEFSKLE KGDVLVCKTT TPLWTTLFQT	780
AGAVITDAGG ILSHSAIAR EYEIPAVVGT KISTNKLKD DIVMVDTNG IVTLLNE	837

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What is claimed is:

1. A method for synthesizing a phenolic phytochemical phosphate derivative, comprising: exposing a phenolic phytochemical to a polypeptide or to a microorganism comprising a nucleic acid sequence encoding the polypeptide for converting the polyphenol phytochemical to its phosphate derivatives;

wherein the polypeptide comprises a homologous protein sequence that is more than 70% identical to the phenolic phytochemical phosphate synthetase (SEQ ID NO: 13); wherein said polypeptide comprises a conserved domain which is based on the phenolic phytochemical phosphate synthetase (SEQ ID NO: 13) and sequentially comprises:

an ATP-binding domain, comprising active catalytic sites of Lys27, Arg102, and Glu282;

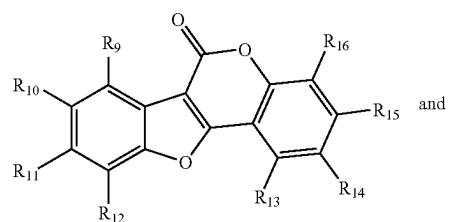
a substrate-binding domain, comprising a conserved motif of DDHHFYIDAMLDKAR (SEQ ID NO: 14), and comprising active catalytic sites of Asp627, His629, and His630;

and a phosphorylated histidine catalytic domain, comprising His795;

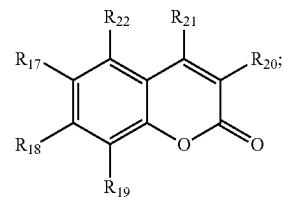
wherein the phenolic phytochemical is selected from the group consisting of the following formulas:

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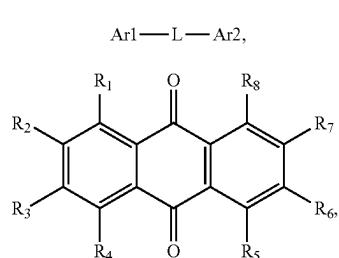
(III)



(IV)

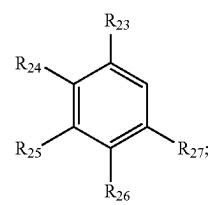


wherein Ar1 is an aryl group of the following formula:

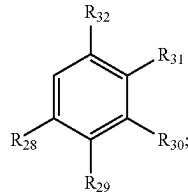


(I)

(II)



Ar2 is an aryl group of the following formula:



L is a linking group comprising 3 to 7 backbone carbon atoms forming a chain linking Ar1 and Ar2 as the case may be, wherein L comprises at least one of a double bond, a carbonyl group and a hydroxyl group;

R1 to R8 are respectively H, (C1-C5)alkyl group, hydroxyl group, OR33, OCH2OR34, OCOR35, COR36, CO2R37, OCH2COOR38, OCH2 (OR39)2, OC=ONHR40, halogen, nitro, amino, NR41R42, cyano group, mercapto group, SR43, S(O)qR44, (C1-C5)chloroalkyl group, (C1-C5)haloalkoxy group, (C2-C6)alkenyl group, (C2-C6)alkynyl group, (C3-C10) cycloalkyl group, (C6-C11)phenyl group or (C7-C12) benzyl group, wherein q is an integral of 1 to 3, and at least one of R1 to R8 is a hydroxyl group;

R9 to R16 are respectively H, (C1-C5)alkyl group, hydroxyl group, OR33, OCH2OR34, OCOR35, COR36, CO2R37, OCH2COOR38, OCH2 (OR39)2, OC=ONHR40, halogen, nitro, amino, NR41R42, cyano group, mercapto group, SR43, S(O)qR44, (C1-C5)chloroalkyl group, (C1-C5)haloalkoxy group, (C2-C6)alkenyl group, (C2-C6)alkynyl group, (C3-C10)cycloalkyl group, (C6-C11)phenyl group or (C7-C12)benzyl group, wherein q is an integral of 1 to 3, and at least one of R9 to R16 is a hydroxyl group;

R17 to R22 are respectively H, methoxy group or hydroxyl group, and at least one of R17 to R22 is a hydroxyl group, or R20 and R21, R17 and R18, R17 and R22, R18 and R19 or their combination are fused to form a (C3-C6)cycloalkyl group with hydroxyl group or a (C6-C10)aryl group with hydroxyl group; R23 to R27 are respectively H, methoxy group or hydroxyl group, and at least one of R23 to R27 is a hydroxyl group;

R28 to R32 are respectively H, methoxy group or hydroxyl group, and at least one of R28 to R32 is a hydroxyl group;

R33 to R34 are respectively (C1-C5)alkyl group, (C1-C5)haloalkoxy group, (C2-C6)alkenyl group, (C2-C6) alkynyl group, (C6-C11)phenyl group or (C7-C12) benzyl group;

R35 is (C1-C5)alkyl group, (C1-C5)haloalkoxy group, (C6-C11)phenyl group or (C7-C12)benzyl group;

R36 is (C1-C5)alkyl group, (C1-C5)haloalkoxy group, (C2-C6)alkenyl group, (C2-C6)alkynyl group, (C6-C11)phenyl group or (C7-C12)benzyl group;

R37 to R40 are respectively (C1-C5)alkyl group or (C1-C5)haloalkoxy group;

R41 and R42 are respectively H, (C1-C5)alkyl group or (C1-C5)haloalkoxy group, one of which is H and the other is not H;

R43 and R44 are respectively H, (C1-C5)alkyl group or (C1-C5)haloalkoxy group.

2. The method according to claim 1, wherein the substrate is a natural or modified curcuminoid, anthraquinones, chalcone, stilbenoid, coumestan or coumarin.

3. The method according to claim 1, wherein the nucleic acid sequence is derived from *Bacillus subtilis*, *Bacillus halotolerans*, *Bacillus atrophaeus*, *Bacillus mojavensis*, *Bacillus velezensis* or *Bacillus amyloliquefaciens*.

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