SECRETORY STRUCTURES, HISTOCHEMICAL AND PHYTOCHEMICAL COMPOUNDS IN THE PHYSIALIS ANGULATA LEAVES (SOLANACEAE) AS A PROSPECT OF MEDICINAL DEVELOPMENT

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Abstract - Physialis angulata L. is the one of Solanaceae families which can be used as traditional medicine. In Indonesia, particularly in North Kalimantan Province, the leaves of P. angulata are used by Dayak Kenyah tribe as herbal medicine to reduces inflammation, relieves pain, prevents ulcers, and reduces fever. This study aims to identify and analyze the secretory structure, histochemical and phytochemical content in the Physialis angulata leaves. Observation of secretory structures by using light microscope showed that P. angulata leaves have the secretory structures in the form of idioblast cells and capitate glandular trichomes. Idioblast cells spread in epidermis. Capitate glandular trichomes on the upper and lower leaf surfaces. Histochemical test indicated that the idioblast cells contains terpenoids compounds. Capitate glandular trichomes has one stalk cell and four head cells containing alkaloid, terpenoid and lipophilic compounds. The GC-MS analysis of the samples shows that thirty phytochemicals were identified in the chloroform extract. Commonly, the components belong to terpenes, including sesquiterpenes, diterpenes and triterpenes. In addition, it is found a group of compounds in the form of esters, fatty acids, alcohols, and steroids. Phytol compounds is one of the main components of the terpenes that has been found. The methanolic extract of the sample was subjected to GC-MS analysis. Thirty-five phytochemicals were identified which include sesquiterpenes, diterpenes, triterpenes, fatty acids, alcohols, and steroids compounds. Those compounds assumed to serve as the infection drugs, so it is possible for drug development in health. In conclusion, the secretory structures were found possible to be used in the development of drug compounds through invitro culture.

Key words - Medicinal plants, Physialis angulata, phyto-compounds, Solanaceae

I. INTRODUCTION

Physialis angulata L. is a member of the Solanaceae family. P. angulata L. species are used in traditional medicines all around the World. It is used to treat asthma, diabetes, hepatitis and malaria in Taiwan [1] and treatment of cancer in Western Africa [2]. In Indonesia, particularly in North Kalimantan Province, the leaves of P. angulata (Fig. 1) are used in traditional medicine by the Dayak Kenyah tribe as an herbal drug to reduce inflammation, relieves pain, prevents ulcers, and reduces fever (Pers comm. Dayak Kenyah tribe, 2017). Various species in solanaceae are used as treat various diseases. They are Schwenkia americana [3], Solanum nigrescens Mart. & Gal., Nicotiana trigonophylla and Solanum rostratum Dunal [4] Commonly, the plants that are used as medicine have secretory structure as place to produced a chemical compounds. The secretory structures in plants include glandular trichomes, idioblast cells and latisifer. Some plants have glandular trichomes are Cordia verbenacea DC. [5], Stachytarpheta angustifolia and Stachytarpheta cayennensis [6]. Secretory structure contains phytochemical compounds. Previous phytochemical study reported that some other plant species of the Solanaceae like Nicotiana plumbaginifolia viv., Datura innoxia Minu, Datura metel Linn. contain alkaloids [7] and Solanum torvum Swartz. contain flavonoid, alkaloid, saponins, steroids and phenol compounds [8]. Increased interest in the research about compounds in cells of medicinal plants has motivated researchers to identify the secretory structure and analyze the histochemistry and phytochemical content in the P. angulata leaves. A scientific study to identify secretory structures that produce metabolite and phytochemical compounds in P. angulata leaves (Solanaceae) would allow phytochemical compounds improvement through tissue culture and cell culture.



Fig. 1. P. angulata growth in Tarakan, North Kalimantan Province (Rupa D, 2017).

II. MATERIAL AND METHODOLOGY

Plant material and Histochemical test

Fresh leaves of P. angulata were collected from Tarakan and Bulungan in North Kalimantan Province. Fresh leaves were collected for the identification structure secretory and histochemistry test. For identification structure secretory, the samples were tranversely sectioned, at 15-20 µm by using a dual purpose microtome (Yamato RV-240), futhermore it observed by using light microscope (Olympus CX21).

For histochemical test, the presence of terpenoid compound in the leaves tissues was identified by soaking leaf section in 5% cupric acetate solution [9]. Alkaloid contents were tested by soaking leaves section in Wagner reagent [10]. The lipophilic compound was tested by sliced sample washed in 70% alcohol for 1 minute then soaked in the 0,03% sudan IV [11].

Preparation plant extract and Phytochemical Analysis

The P. angulata leaves were dried using oven at 60^0C for three days, after that the leaves were grinded into powder. Respectively, the leaf powder of the P. angulata were exhaustively extracted with methanol (1:7) and chlorofom (1:7). Phytochemical analysis using by GC-MS. For GC-MS analysis, each 2 mg of methanol dan chlorofom extraxct was filled into the chamber in the GC-MS instrument (Shimadzu-QP2010). Furthermore, the samples of chemical component was identified in the process of condition: pyrolysis temperature 300^{o}C, oven temperature GC 50^{o}C, injector temperature 280^{o}C, and helium carrier gas, inter surface temperature 280^{o}C and ion source temperature 200^{o}C.

The spectrogram mass resulted from those processes were then calculated by the instrument automatically based on the similarity of m/z pattern through mass spectrogram in the instrument database (NIST and Wiley).

III. RISULTS

Secretory Structure and Histochemical of P. angulata leaves

In P. angulata leaves, we observed two types of secretory structures i.e. capitate glandular trichomes and idioblast cells (Fig. 2). Capitate glandular trichomes are spread over the upper (Adaxial) and lower (Abaxial) leaves surface. They have one stalk cell and four head cells containing alkaloid, terpenoid and lipophilic compounds, while idioblast cells spread over the epidermis containing terpenoid compounds, but there is no presence of terpenoid and lipophilic compounds.

Phytochemical Compounds

GC-MS analysis of the chloroform extract of P. angulata leaves resulted in the identification of thirty compounds (Fig. 3 and Table 1). Commonly, components that are belong to terpenes, including sesquiterpenes, diterpenes and triterpenes, while fatty acid, alcohol, steroid etc. The results revealed that Phytol (29,91%) was found to be major component followed by Hexadecanoic acid (CAS) Palmitic acid (11,87%).

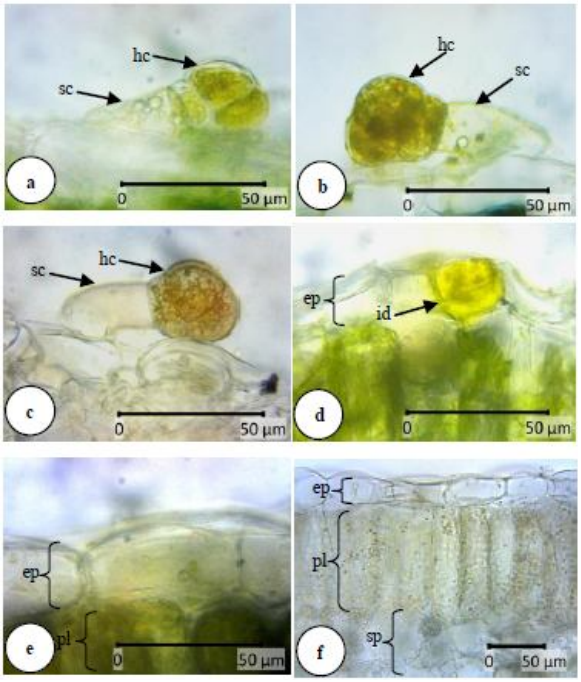


Fig. 2. Capitate glandular trichomes (a,b & c) and idioblast cell (d) in the P. angulata leaves. Histochemical test using cupric acetate for terpenoid (a and d), Wagner test for alkaloid (b and e), Sudan IV test for lipophilic substance (c and f). sc: stalk cells, hd: head cells, ep: epidermis tissue, pl: palisade tissue, id: idioblast cell and sp: spongy tissue.

Thirty-five compounds were detected in methanolic extract of P. angulata leaves (Fig. 4 and Table 2). which include sesquiterpene, diterpene, triterpene, alkaloid, fatty acid, alcohol, and steroid compounds. The main constituents are: 9,12,15-Octadecatrienoic acid,methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate (13,36), Hexadecanoic acid (CAS) Palmitic acid (12,82%) and 9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid (10,81%). Alkaloid compounds in the form Pyridine,3-(1-methyl-2-pyrrolidinyl)-,(S)-(CAS)Nicotine$$1-methyl-2-(3-pyridyl)pyrro were found in methanolic extract, but not precense in the chlorofom extract. Classes of compounds that are often detected are fatty acids and terpene compounds. But terpene compounds often is detected. Fatty acid that have been detected i.e. Nonanoic acid, 9-oxo-, ethyl ester, Dodecanoic acid (CAS) Lauric acid, Hexadecanoic acid (CAS) Palmitic acid, Hexadecanoic acid, Ethyl ester (CAS) Ethyl palmitate, 9,12-Octadecadienoic acid (Z, Z) , Methyl ester (CAS) Methyl linoleate etc.

But also were found steroid compounds i.e Ergosta-5,24-dien-3-ol, acetate, (3.beta.) - (CAS) Ergosta-5,24-dien-3.beta.-o, and 17- (1.5-dimethyl-hexyl) 10,13-dimethyl-1,7,8,9,10,11,12,13,14,15,16.

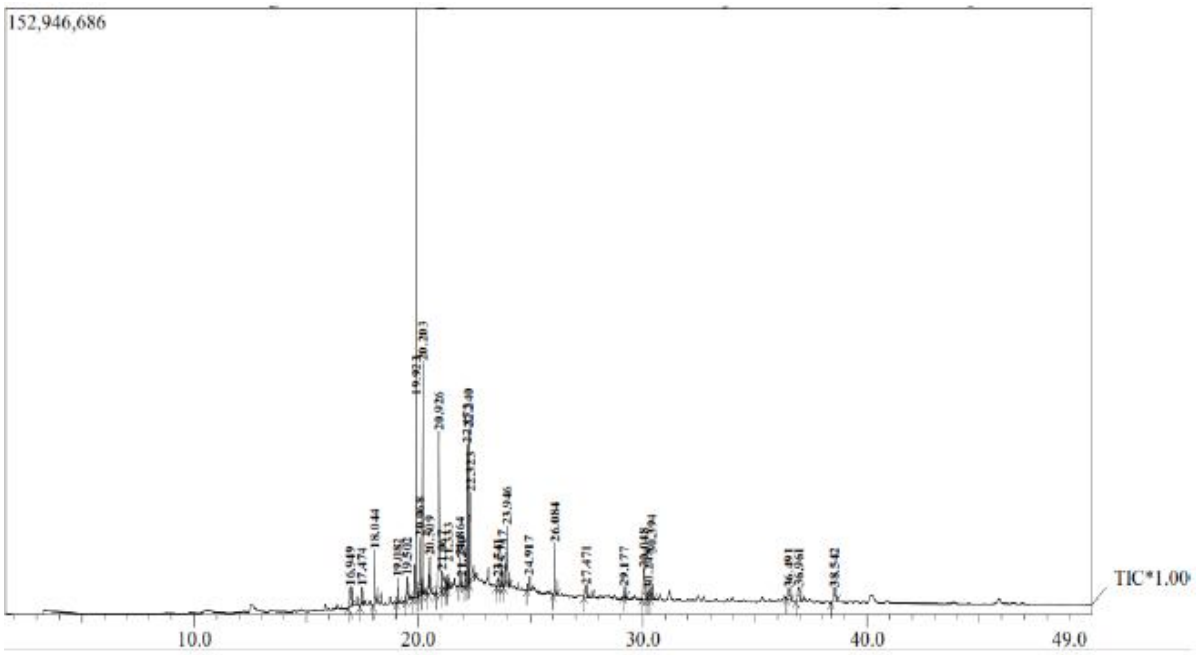


Fig 3. GC-MS Chromatogram of the chloroform extracts of P. angulata leaves.

Peak #	R.Time	Area	Conc%	Group	Peak Report TIC Name
1	16.949	26307152	1.90	Ketone	2(1H)-Naphthalenone, 4a,5,6,7,8,8a-hexahydro-4a-methyl-, trans-
2	17.474	9243471	0.67	Fatty acid	Nonanoic acid, 9-oxo-, ethyl ester
3	18.044	41708871	3.01	Fatty acid	Dodecanoic acid (CAS) Lauric acid
4	19.082	12227417	0.88	Alcohol	1-Hexadecanol (CAS) Cetal
5	19.502	14941956	1.08	Fatty acid	9-Octadecenoic acid (Z)- (CAS) Oleic acid
6	19.923	294841210	21.29	Diterpene	Phytol
7	20.068	40239398	2.91	Alkane	3-Eicosyne
8	20.203	100726661	7.27	Diterpene	Phytol
9	20.509	31073298	2.24	Beta springere	(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene
10	20.926	164358131	11.87	Fatty acid	Hexadecanoic acid (CAS) Palmitic acid
11	21.067	30596334	2.21	Sesquiterpene	beta.-Farnesene
12	21.250	12700888	0.92	Sesquiterpene	trans-Caryophyllene
13	21.333	8900253	0.64	Sesquiterpene	Farnesol
14	21.864	18884128	1.36	Diterpene	Phytol
15	22.173	97560692	7.05	Fatty acid	9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid
16	22.240	118535031	8.56	Alcohol	9,12,15-Octadecatrien-1-ol (CAS) OCTADECA-9,12,15-Trien-1-ol
17	22.323	100752877	7.28	Ester	Octadecanoic acid, ethyl ester (CAS) Ethyl stearate
18	23.541	8217488	0.59	Alkanoic acid	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate
19	23.747	10682188	0.77	Cyclohexane	Cyclohexane, eicosyl- (CAS) 1-Cyclohexyleicosane
20	23.946	59343512	4.29	Fatty acid	Hexadecanoic acid, ethyl ester (CAS) Ethyl palmitate
21	24.917	11802224	0.85	Fatty acid	Nonadecanoic acid, ethyl ester
22	26.084	33954267	2.45	Ester	Ethyl ester of docosanoic acid
23	27.471	13827950	1.00	Ester	Ethyl ester of docosanoic acid
24	29.177	8150140	0.59	Fatty acid	Nonadecanoic acid, ethyl ester

25	30.048	30234239	2.18	Triterpene	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- (CAS) Squalene
26	30.248	10095907	0.73	Diterpene	Geranyl linalool isomer B
27	30.394	12028554	0.87	Sesquiterpene	Farnesol
28	36.491	18040474	1.30	Cholesterol	17-(1,5-DIMETHYL-HEXYL)-10,13-DIMETHYL-1,7,8,9,10,11,12,13,14,15,16,
29	36.961	21041686	1.52	Hexatriacontane	Hexatriacontane (CAS) n-Hexatriacontane
30	38.542	23582664	1.70	Steroid	Ergosta-5,24-dien-3-ol, acetate, (3.beta.)- (CAS) Ergosta-5,24-dien-3.beta.-o
		1384599061	100.00		

Table 1. Components detected in chloroform extract of P. angulata leaves by GC-MS analysis.

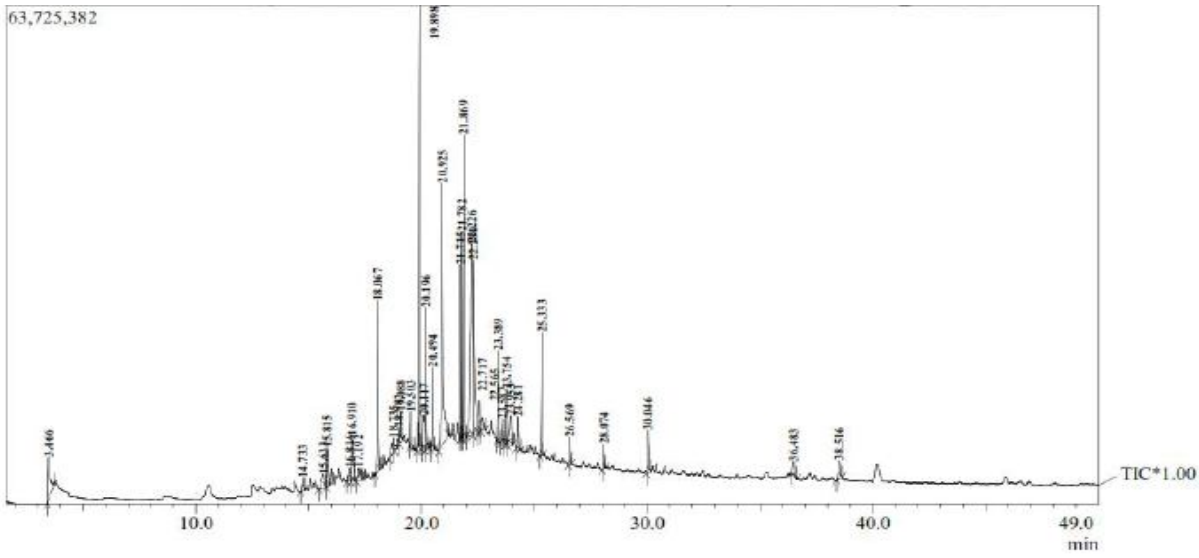


Fig 4. GC-MS Chromatogram of the Methanolic extracts of P. angulata leaves

Peak#	R.Time	Area	Conc %	Group	Peak Report TIC Name
1	3.466	34933104	2.79	Cyclopropane	Cyclopropane, 1,1-dibromo-2-chloro-2-fluoro- (CAS) 1,1-dibromo-2-chlo
2	14.733	8761650	0.70	Alcohol	Phenol, 2,3-dimethyl- (CAS) 2,3-Dimethylphenol
3	15.633	15427463	1.23	Furan	2-Butanone, 4-(5-methyl-2-furanyl)- (CAS) 1-(5'-methyl-2'-furyl)butan-
4	15.815	17500427	1.40	Alcohol	Phenol, 2-(2-propenyl)- (CAS) o-Allylphenol
5	16.816	9229796	0.74	Alkaloid	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)- (CAS) Nicotine $$ 1-methyl-2-(3-pyridyl)pyrro
6	16.910	31759084	2.54	Aldehide	4-Hydroxymethylbenzaldehyde
7	17.192	14514461	1.16	Cyclohexanone	Cyclohexanone, 2-(1-hydroxyethyl)- (CAS) 3-methylene-bicyclo[3.2.1]o
8	18.067	75167277	6.01	Fatty acid	Dodecanoic acid (CAS) Lauric acid
9	18.735	13794034	1.10	Terpene	Megastigmatrienone 4
10	18.982	11687989	0.93	Alcohol	1-Naphthalenol, 3-methyl- (CAS) 3-Methyl-1-naphthol
11	19.088	8768368	0.70	Alkene	1-Pentadecene (CAS) Pentadec-1-ene
12	19.503	14369860	1.15	Fatty acid	Tetradecanoic acid (CAS) Myristic acid
13	19.898	93639137	7.49	Diterpene	Neophytadiene
14	20.117	27740836	2.22	Ketone	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)- $$
15	20.196	37294174	2.98	Alkene	1-Hexadecyne
16	20.494	26118742	2.09	Fatty acid	Pentadecanoic acid, 14-methyl-, methyl ester (CAS) Methyl 14-methyl-p
17	20.925	161501950	12.92	Fatty acid	Hexadecanoic acid (CAS) Palmitic acid
18	21.715	44541097	3.56	Fatty acid	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS) Methyl linoleate
19	21.782	42008587	3.36	Fatty acid	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate
20	21.869	84024810	6.72	Diterpene	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phytol
21	22.226	135174055	10.81	Fatty acid	9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid
22	22.290	126212354	10.10	Fatty acid	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate
23	22.565	19343703	1.55	Fatty acid	9-Octadecenamide, (Z)- (CAS) Oleoamide
24	22.717	10792419	0.86	Sesquiterpene	Bicyclogermacrene

25	23.389	25157810	2.01	Fatty acid	Eicosanoic acid, methyl ester (CAS) Arachidic acid methyl ester
26	23.587	10527797	0.84	Fatty aldehydes	9-Octadecenal, (Z)- (CAS) Cis-octadec-9-enal
27	23.754	16275388	1.30	hexane	Cyclohexane, eicosyl- (CAS) 1-Cyclohexyleicosane
28	23.953	20436002	1.63	Alcohol	Cyclopropanemethanol, 2-methyl-2-(4-methyl-3-pentenyl)-
29	24.281	12409212	0.99	Fatty acid	Triacontanoic acid, methyl ester (CAS) Methyl triacontanoate
30	25.333	35854817	2.87	Fatty acid	Docosanoic acid, methyl ester (CAS) Methyl behenate
31	26.569	9271108	0.74	Fatty acid	Tricosanoic acid, methyl ester (CAS) Methyl tricosanoate
32	28.074	9530890	0.76	Fatty acid	Tetracosanoic acid, methyl ester (CAS) Methyl lignocerate
33	30.046	21020296	1.68	Triterpene	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- (CAS) Squalene
34	36.483	9384949	0.75	Steroid	17-(1,5-dimethyl-hexyl)-10,13-dimethyl-1,7,8,9,10,11,12,13,14,15,16,
35	38.516	16032413	1.28	Steroid	Ergosta-5,24-dien-3-ol, acetate, (3.beta.)- (CAS) Ergosta-5,24-dien-3.beta.-o
		1250206059	100.00		

Table 2. Components detected in methanolic extract of P. angulata leaves by GC-MS analysis

DISCUSSION

Species in solanaceae family very much researched as medicinal plant. They are Solanum xanthocarpum , Physalis minima Linn, Solanum nigrum Linn., and Withania somnifera Dunnal. They contain alkaloids compound [7]. Dichrostachys cinerea and Indigofera pulchra were possess some potential anti-inflammatory properties. The content of the compounds in the plant is saponins [3], futhermore phenolic and flavonoids content in Solanum nigrum and Solanum lyratum. Solanum lyratum as antiinflammatory [12].

Based on identification of secretory structure were found glandular trichomes and idioblast cells in physialis leaves. These structures play a role in the accumulation of chemical compounds. Histochemical tests that terpene, alkaloid and lipophilic compounds were detected in the structure. While the analysis of leaf extraction detected terpene compounds such as phytol, beta.-Farnesene, trans-Caryophyllene, farnesol, 2,6,10,14,18,22- Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, geranyl linalool isomer B, megastigmatrienone 4, neophytadiene, and bicyclogermacrene and alkaloid in the form pyridine,3-(1-methyl-2-pyrrolidinyl)-,(S)-. Based on this results, it can be concluded that the compounds accumulates in the secretory structure. Terpene have been reported synthesized in glandular trichomes of Humulus lupulus L. [13], Solanum habrochaites [14] and Nocotiana tabacum [15]. Other than, that alkaloids, tannins, lipophilic and hydrophilic compounds are secreted in N. tabacum glandular trichomes [15].

Phytol have the highest percentage content. Phytol have been reported to be found in other species such as Andrographis paniculata [16], Eupatorium odoratum [17] and Gracilaria edulis [18]. Leaf extract of A. paniculata and E. odoratum have an activity as antimicrobial, antioxidant [17] [16] and anticancer (MCF-7) activities [16] .Phytol have been reported to possess a wide range of biological activities. Phytol compounds at different concentrations capable reduced carrageenan-induced paw edema [19] and anti-proliferation activity against MCF-7 cell lines [18]. Secretory structures and compounds that have been found as prospects in drug development and they are highly useful targets for plant metabolic engineering.

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