Supporting information

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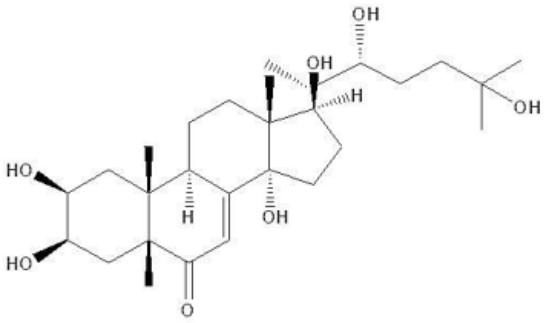
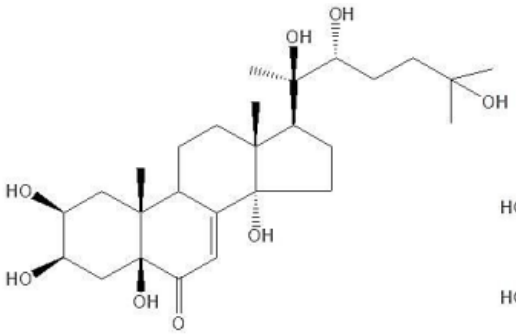
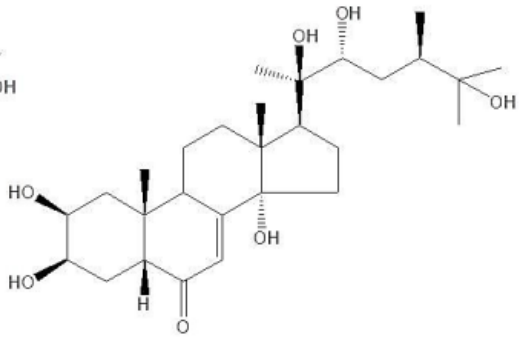


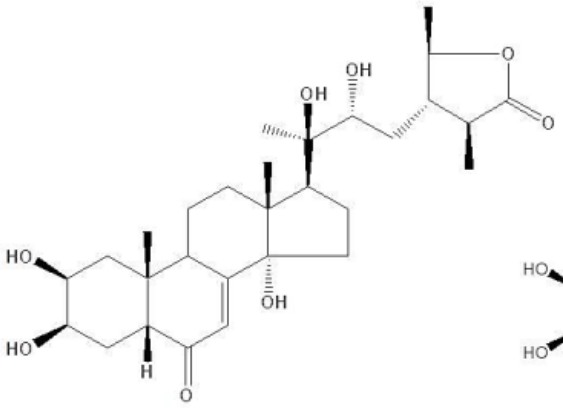
20-hydroxyecdysone



Polypodine B



Makisterone A



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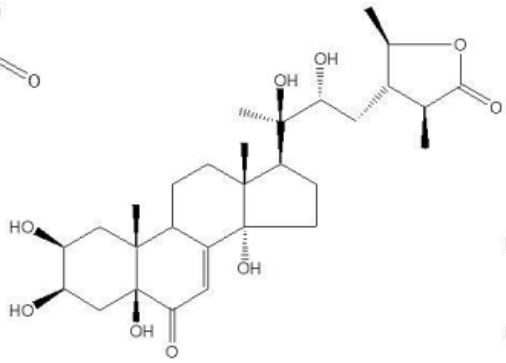

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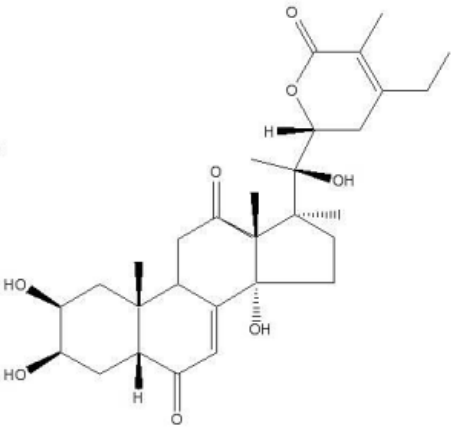

Ajugalactone

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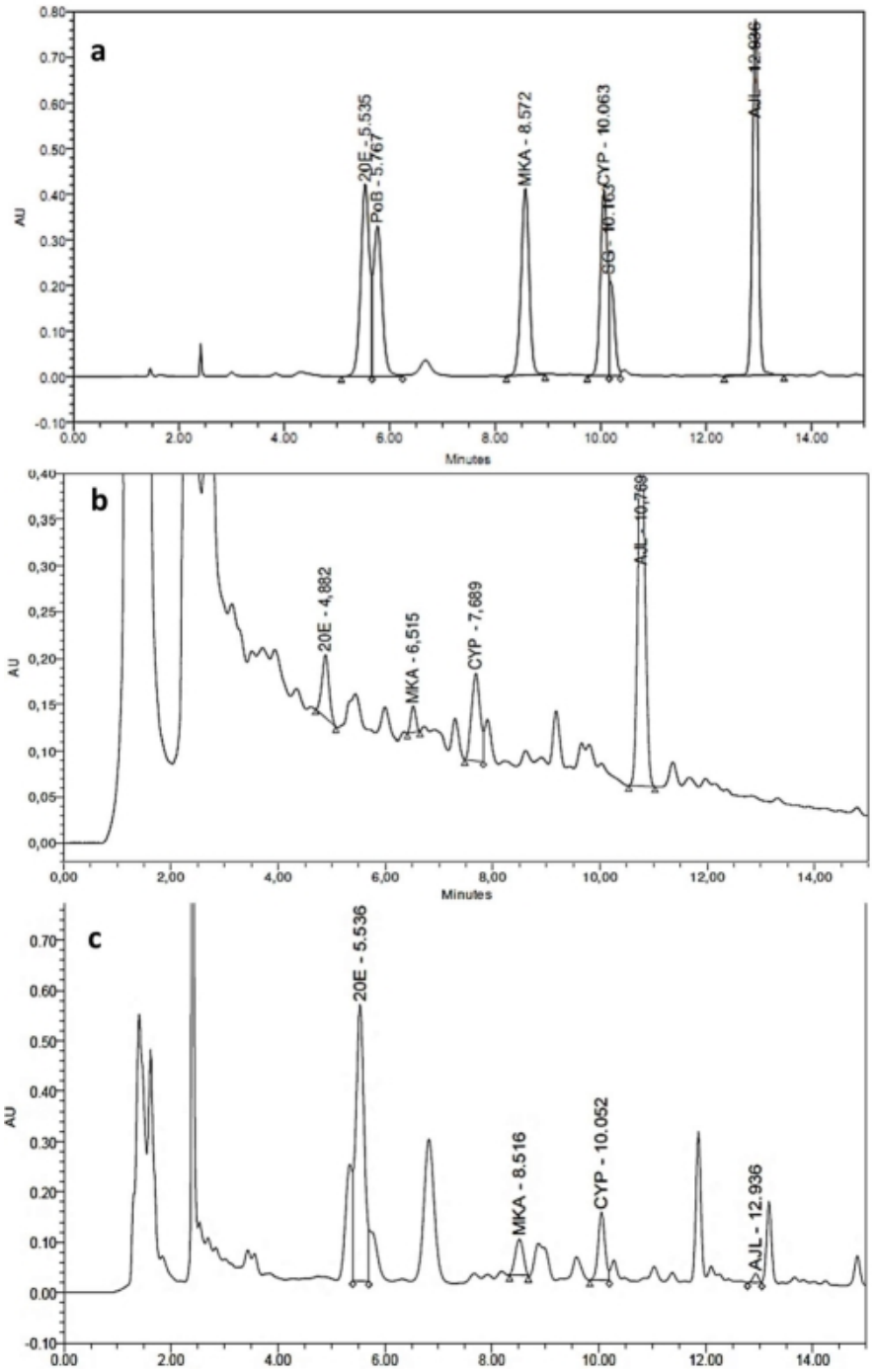
Supplementary Fig. 2 RP-HPLC Chromatographs representing elution pattern of phytoecdysteroids in different samples of *A. bracteosa*. a, Elution of six standard phytoecdysteroids. **b,** phytoecdysteroids profiling in pPCV002-ABC transgenic line 1 (ABC1). **c,** phytoecdysteroids pattern of elution in transgenic hairy root line (A1).



Supplementary Table 1 Media used for the optimization of hairy root induction, stabilization and steady growth in *A. bracteosa*.

Medium	Composition
First phase media (hairy root induction ≥ 10 days)	MS/B5+ Claforan 500 mg/L
	SH+ Claforan 500 mg/L
Second phase media (hairy root proliferation ≥ 30-35 days)	0.5MS+ Claforan 500 mg/L
	0.5MS+IBA+ Claforan 500 mg/L
	0.5 B5+ Claforan 500 mg/L
	0.5 B5+IBA+ Claforan 500 mg/L
	SH+ Claforan 500 mg/L
Third phase medium (stable growth and maintainability)	MS+ Claforan 250 mg/L
	0.5MS+ Claforan 250 mg/L
	B5+ Claforan 250 mg/L
	0.5 B5+ Claforan 250 mg/L
	SH+ Claforan 250 mg/L

After one month of culturing of hairy roots in third phase medium, claforan was removed from it.

Supplementary Table 2 Effect of medium on induction of hairy roots in *A. bracteosa*.

Media	Strain	Explants	No. of roots	Explants producing roots	Root induction (days)	Branching (%)
MS/B5	LBA-9402	839	49	34	13	40
	A4	415	33	23	12	55
	Arqua1	262	12	8	9	60
SH	LBA-9402	60	289	31	7	90
	A4	20	78	5	8	100

Supplementary Table 3 Effect of medium on proliferation of hairy roots in *A. bracteosa*.

Strain	Media	Growth status of roots
LBA-9402	0.5B5+IBA	Secondary branches, increase in length, few produce callus
	0.5B5	Single root, no ramification, very slow growth
	0.5MS	Single root, no ramification, very slow growth
	0.5MS+IBA	More increase in length than width, ramification, some produce callus
	SH	No ramification, callus induction
A4	0.5B5+IBA	Ramification, length increase, some generate callus by increasing width
	0.5B5	No growth, no ramification
	0.5MS	No growth, no ramification
	0.5MS+IBA	Ramification, increased in width and swollen, length also increased
	SH	Pale yellow, dying
ARqua1	0.5B5+IBA	Swollen callus, spiny
	0.5B5	No growth, no ramification, greyish
	0.5MS	Dead
	0.5MS+IBA	Dying, no growth
	SH	Surviving

Supplementary Table 4 Effect of medium on stable growth and maintainability of hairy roots in *A. bracteosa*.

Media	Growth status of roots
B5	Bit swollen, broader, thick, ramification but less increase in length
MS	Callus like, beaded appearance, no increase in length but increase in width
0.5B5	Normal hairy roots, ramification, increase in length found, few are thick
0.5MS	Multiple ramification, densely hairy, increase in length, few are thick
SH	No increase, pale yellow roots, dying

Supplementary Table 5 Effect of explants' origin on hairy root induction and its attributes in *A. bracteosa*.

	Strain	Explants	No. of roots	Explants producing roots	Root induction (days)	Branching
In vitro	LBA-9402	634	22	15	14	No
	A4	245	12	15	13	No
	Arqua1	204	9	5	8	No
Ex vitro	LBA-9402	265	301	41	11	Yes
	A4	190	99	16	12	Yes
	Arqua1	58	3	3	11	Yes

Supplementary Table 6 Attributes of genes screened by PCR and semi-quantitative RT-PCR analysis in transgenic *A. bracteosa*.

Vector	Gene	Sequence	Size	T_m
pPCV002-ABC (PCR)	*rolA*	*rolA*1:5'-AGAATGGAATTAGCCGGACTA-3' *rolA*2:5'-GTATTAATCCCGTAGGTTTGTT-3'	308 bp	53°C
	rolB	*rolB*1:5'-GCTCTTGCAGTGCTAGATTT-3' *rolB*2:5'-GAAGGTGCAAGCTACCTCTC-3'	779 bp	55°C
	rolC	*rolC*1:5'-GAAGACGACCTGTGTTCTC-3' *rolC*2:5'-CGTTCAAACGTTAGCCGA TT-3'	540 bp	54°C
	npt-II	*npt-II*-1:5'AAGATGGATTGCACGCAGGTC3' *npt-II*-2:5'GAAGAACTCGTCAAGAAGGCG3'	780 bp	54°C
pRI A4, LBA-9402 and ARqua1 (PCR)	*rolC*	*rolC*1:5'-TAACATGGCTGAAGACGACC-3' *rolC*2:5'-AAACTTGCACTCGCCATGCC-3'	534 bp	60°C
	virD1	*virD1*-1:5'-ATGTCGCAAGGCAGTAAGCCC-3' *virD1*-2:5'-GAAGTCTTTCAGCATGGAGCA-3'	438 bp	56°C
pPCV002-ABC, *pRI A4* and *LBA-9402* (SQ-RT-PCR)	*rolC*	*rolC*1:5'-CTGTACCTCTACGTCGACT-3' *rolC*2:5'-AAACTTGCACTCGCCATGCC-3'	363 bp	62°C
	actin	*actin*1:5'-ATCAGCAATACCAGGGAACATAGT-3' *actin*2:5'-AGGTGCCGTGAGGTCTTGTTCC-3'	160 bp	60°C

PCR conditions were fixed except for annealing temperature. They were; 5 minutes 95°C, followed by 35 cycles of 35 seconds at 95°C, 35 sec for primers annealing (temperature given in table), and finally an extension of 10 min at 70°C.

Supplementary Table 7 Analysis of Variance (ANOVA) of *pPCV002-ABC* transformed intact plants (a) and transgenic hairy root lines (b) using 2-Factor Complete Randomized Design.

(a)

SOURCE	DF	SS	MS	F-value	*P* value
Transgenic plants	7	28379204	4054171.944	2794.538	***
Phytoecdysteroids	3	2628070	876023.351	603.8423	***
Transgenic plants × phytoecdysteroids	21	7420919	353377.094	243.5826	***
Error	64	92847.91	1450.749		
TOTAL	95	38521041			

Coefficient of Variation: 4.68%

(b)

SOURCE	DF	SS	MS	F-value	*P* value
Transgenic hairy roots	58	22012543.866	379526.618	590.6268	***
Ecdysteroids	3	39953845.301	13317948.434	20725.6527	***
Transgenic hairy roots × phytoecdysteroids	174	18722812.986	107602.373	167.4529	***
Error	472	303299.093	642.583		
TOTAL	707	80992501.246			

Coefficient of Variation: 6.71%.

ANOVA of transgenic hairy root lines was done against 59 established root clones. Out of them, 11 elite lines are mentioned here for phytoecdysteroids profiling and among those eleven, 9 hairy root clones which were obtained from the infection of LBA-9402 were studied further for expression analysis. DF, degree of freedom; SS, sum of squares; MS, means square.