HPLC***Calendula officinalis* L.**

/ /

(2013/ 8/ 19 2013/ 4 /28)

MS

2,4-D 0.1 ± 5.7 pH / 6 / 30 ÷
 / (0.5 0.2 0.1 0.0)
 BA / 0.1 NAA / (2.0 1.5 1.0 0.5 0.0) MS

High

13.04 : (HPLC) Performance Liquid Chromatography

BA / 0.1 NAA / 1.0 ÷
 20.50
 BA / 0.1 NAA / 1.5 ÷
 / 30.02

BA / 0.1 NAA / 1.5 ÷
 / 139.28

BA / 0.1 NAA / 2.0 ÷

Calendula officinalis L. :

Qualitative and Quantitative Determination of Salicylic acid and Resorcinol in Callus of *Calendula officinalis* L. Using HPLC Technique

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ABSTRACT

Parts of leave of *Calendula officinalis* plant before and after blooming were cultured on MS medium supplemented with 30 g / l scores, 6 g / l agar, pH was 5.7± 0.1, in addition to 2,4-D at (0.0 , 0.1 , 0.2 , 0.5) mg/ l for callus induction, produced callus recultured on medium supplemented with (0.0, 0.5, 1.0, 1.5, 2.0) mg/ l NAA interaction with 0.1 mg/ l BA for three times three weeks for each one. Salicylic acid and Resorcinol were stimulated and identified in callus using High Performance Liquid Chromatography (HPLC) method. The data indicate: highest fresh weight of callus 13.04 gm achieved from cultured parts of leaves before flowering on medium supplemented with 1.0 mg/ l NAA and 0.1 mg/ l BA after nine weeks, and the highest fresh weight of callus

20.50 g obtained from cultured parts of leaves during flowering on medium supplemented with 1.5 mg/ l NAA and 0.1 mg/ l BA after nine weeks, highest quantity of salicylic acid 30.02 mg/gm dry weight was obtained from callus produced from parts of leaves before flowering cultured on MS medium supplemented with 1.5 mg/ l NAA and 0.1 mg/ l BA after six weeks, highest quantity of Resorcinol 139.28 mg/ gm dry weight was obtained from callus produced from parts of leaves took during flowering cultured on MS medium supplemented with 2.0 mg/l NAA and 0.1mg/ l BA after six weeks.

Keywords: *Calendula officinalis* L., Salicylic acid, Resorcinol, Callus.

Asteraceae

Calendula officinalis L.

.(1999 1986)

(John and Harold, 2004)

.(Khare , 2004 Itcolor and Major, 1993)

()

.(Tripathi and Tripathi, 2003)

Flavonoids Polyphenols Carotenoids

(Preethi *et al* ., 2006 Bernatoniene *et al.*, 2011)

(Medina *et al.*, 2006) Anti-genotoxic

Anti-viral Anti-inflamaory

()

(Bissa and Bohra, 2012)

(Muley *et al.*, 2009) Anti-HIV ()

.(Amoian *et al.*, 2010)

Calenduladiol-3-myristate

Rutin

Narcissin

Comarine

Flavonoids

Volatile oil

arginine, alanine aspartic

glutamic, acid histidine, valine, acid

.(Muley *et al.*, 2009) Calendin

Loliolide

.....

vanillic acid, syringic acid, sinapic acid,
gallic acid, coumaric acid, ferulic acid, anisic acid, rosmarinic acid, salicylic acid, cinnamic acid
Gallic acid HPLC gentsic acid, chlorogenic acid

(Khalil *et al.*, 2007) (Wen *et al.*, 2005) Cinnamic acid

Hydroxybenzoic acid Resorcinol

Coumarin Cinnamic acid P-coumric acid Salicylic acid Chlorogenic acid

(Kaskoniene *et al.*, 2011)

/ 0.2

/ 0.05

Hayat and Ahmad, 2007)

(Sakhanokho and Kelley, 2009;

.(Verberne *et al.*, 1999 ;Yalpani *et al.*, 1993) pathogenesis-related proteins

Methyl

.(Molinari, 2007 Hayat and Ahmad, 2007) Salicylate

.(Shuzo *et al.*, 2005 ; Krumenacker *et al.*, 1995) UV

.(Zhou and Wu, 2006)

MS

2-4,D

MS

Salicylic

BA / 0.1

NAA

.HPLC

Resorcinol acid

15

/ 30 MS 1.0 (Bertoni *et al.*, 2006)
 0.1 ±5.7 pH / 6
 / (0.5, 0.2, 0.1, 0.0) 2,4-D

MS

BA / 0.1 NAA / (2.0, 1.5, 1.0, 0.5, 0.0)

:

24 ° 51 (Oven)

%70 100
 ° 55-50 48 Hotplate magnetic stirrer
 ° 4 Whatman No.1
 10 ° 70

50

30 ° 80

.(Harborne, 1973)

0.025 Resorcinol Salicylic acid

Shimadzu corporation HPLC 250

Macherey-) USA / 2010 A

(Octadecyl silane) ODSI () (Nagel, C18-LC, 150x4 , 6 mm

° 35 Silica gel

5 2 / 2 flow rate water %10 acetonitrile %90

320

:

x

$$\frac{(\quad)}{(\quad)} \times \frac{(\quad)}{(\quad)} = (\quad / \quad)$$

(Behbahani *et al.*, 2011)

.....

Micro-kieldahl

.A.O.A.C. (2002)

-

20

100

20

²

/

1.04

121

8

16

1 ± 25

C R D

.

3000

%5

.(1999

)

2,4-D

(1)

2,4-D

/

0.1

1.14

1.59

2,4-D

/

0.2

.2,4-D

/

0.5

0.1

.(1990

)

()

:1

Calendula officinalis

2,4-D

MS

()		2,4-D / MS
0.0b	0.11c	0.0
1.19a	1.14a	0.1
1.59a	0.85b	0.2
1.08a	0.79b	0.5

. %5

•

BA

/

0.1

NAA

(2)

2,4-D

/

0.1

÷

MS

0.1

NAA

/

1.0

5.1

BA

/

BA

/

0.1

NAA

/

1.0

1.5

7.14

9.31

/

1.0

0.1 NAA / 1.5 13.04 BA / 0.1 NAA
12.86 BA /

.(Hartmann *et al.*, 2002)

BA / 0.1 NAA :2

***Calendula officinalis* L.**

()			/ NAA
7.41b	2.23b	1.38c	0.0
6.97b	4.43b	2.63b	0.5
13.04a	7.14a	5.10a	1.0
12.86a	9.31a	3.62b	1.5
5.57b	2.34b	1.23c	2.0

.% 5

*

BA / 0.1 NAA (3)

2,4-D / 0.2 MS

NAA / 1.5

20.50 15.85 2.81

BA / 0.1

NAA 3 2

(2002 Colli and Kerbaury,1993)

.(1990)

BA / 0.1 NAA :3

Calendula officinalis

()			/ NAA
4.07c	2.72c	1.11b	0.0
8.55	4.44c	1.79b	0.5
9.70b	8.40b	1.86b	1.0
20.50a	15.85a	2.81a	1.5
6.19bc	3.62c	1.81b	2.0

. % 5

*

: HPLC Resorcinol Salicylic acid

Resorcinol Salicylic acid

(Retention time)

Salicylic acid

(1) (3090218)

(1.311)

(1.436)

Resorcinol

.(2) (121049)

Salicylic acid

()

:1

Resorcinol

()

:2

(4)

BA / 0.1

NAA / 1.5

MS

(30.02) Salicylic acid

(9278596)

(1.309)

(3)

/

1.5

/ (0.31) Salicylic acid

(5)

% 2.450

(1.272) BA / 0.1 NAA /
 BA / 0.1 NAA / 0.5 (4) (97716)
 (5) (1502237) (1.465)

/ 124.1 Resorcinol
 (5) %(2.765)
 BA / 0.1 NAA / 1.5
 (1.487) Resorcinol / (18.30)
 .(4) (221598)

Salicylic / :4
Resorcinol acid
 / 0.1 NAA MS *Calendula officinalis* L.
 BA

Resorcinol			Salicylic acid			NAA /	()
/		Ret. Time	/		Ret. Time		
51.67	625568	1.487	-	-	-	0.0	3
42.12	509905	1.449	-	-	-	0.5	
40.79	493852	1.493	1.31	407008	1.306	1.0	
18.30	221598	1.487	0.31	97716	1.272	1.5	
112.16	1357716	1.448	0.85	265582	1.267	2.0	
34.83	421670	1.468	0.46	144249	1.312	0.0	6
124.1	1502237	1.465	-	-	-	0.5	
66.45	804396	1.475	0.62	190482	1.319	1.0	
-	-	-	30.02	9278596	1.309	1.5	
-	-	-	16.72	5168341	1.346	2.0	
22.44	271646	1.482	0.47	147019	1.343	0.0	9
76.26	923166	1.475	1.60	496202	1.356	0.5	
-	-	-	25.41	7854530	1.347	1.0	
20.58	249229	1.490	0.43	133968	1.310	1.5	
22.27	269661	1.499	0.53	165298	1.278	2.0	
	121049	1.436		3090218	1.311		

Calendula :5
 BA / 0.1 NAA MS *officinalis* L.

			/ NAA
9	6	3	
3.062	1.837	1.531	0.0
1.837	2.765	1.531	0.5
2.450	1.837	1.837	1.0
1.837	2.450	1.531	1.5
1.531	2.450	2.450	2.0

.....

Resorcinol Salicylic acid () :3

1.5 MS *Calendula officinalis*

6 BA / 0.1 NAA

Resorcinol Salicylic acid () :4

1.5 MS *Calendula officinalis*

3 BA / 0.1 NAA /

Resorcinol Salicylic acid () : 5

0.5 MS *Calendula officinalis*

6 BA / 0.1 NAA /

(6)

Salicylic BA / 0.1 NAA / 0.0 MS

(1.359) / (2.81)acid

(6) (868439)

(7) %2.450

/ :6

Resorcinol Salicylic acid

NAA MS *Calendula officinalis* L.

BA / 0.1

Resorcinol			Salicylic acid			NAA / ()	
/		Ret. Time	/		Ret. Time		
81.09	981637	1.476	-	-	-	0.0	3
95.43	1155216	1.466	-	-	-	0.5	
48.99	593090	1.488	-	-	-	1.0	
36.26	438941	1.429	1.13	351709	1.306	1.5	
63.50	768750	1.510	0.63	197754	1.249	2.0	
107.45	1300761	1.466	2.81	868439	1.359	0.0	6
130.18	1575828	1.466	-	-	-	0.5	
105.10	1272242	1.477	-	-	-	1.0	
71.36	863856	1.488	-	-	-	1.5	
139.28	1686030	1.414	-	-	-	2.0	
69.41	840231	1.482	-	-	-	0.0	9
61.20	740853	1.480	-	-	-	0.5	
93.97	1137595	1.508	-	-	-	1.0	

.....

69.38	839921	1.495	-	-	-	1.5	
73.00	883662	1.527	-	-	-	2.0	
	121049	1.436		3090218	1.311		

BA / 0.1 NAA / 2.0
(1686030) (1.414)
(7) / (139.28) Resorcinol
(7) % 3.062
Resorcinol (1990)
(1.429) BA / 0.1 NAA / 1.5
(8) / (36.26) (438941)

.(Charlwood *et al.*, 1990)

.(2002)

Calendula :7

BA / 0.1 NAA MS *officinalis* L.

			/ NAA
9	6	3	
1.837	2.450	2.143	0.0
2.450	2.450	2.450	0.5
2.143	1.837	1.837	1.0
2.143	1.531	2.450	1.5
1.837	3.062	1.531	2.0

Resorcinol Salicylic acid () :6
 0.1 MS *Calendula officinalis*
 6 BA /

Resorcinol Salicylic acid () :7
 2.0 MS *Calendula officinalis*
 6 BA / 0.1 NAA /

Resorcinol Salicylic acid () :8
 1.5 MS *Calendula officinalis*
 3 BA / 0.1 NAA /

" (2002)

21 " (2002)

" (1986)

" (1999)

.167 -166 1 :

" (1990)

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