Kantham Srinivas. *et al.* / *International Journal of Phytopharmacology,* 1(2), 2010, 97-102.

ISSN 0975 – 9328

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

ANTI-MITOTIC ACTIVITY OF EMBELIN DERIVATIVES

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ABSTRACT

Identifying the active chemical ingredients of ancient medicines and the molecular targets of those ingredients is an attractive therapeutic objective. Embelin is isolated from berries of *embelia ribes* using hexane and crystallized from benzene. Series of Embelin derivatives, 2-hydroxy-5-substituted-3-undecylcyclohexa-2,5-diene-1,4-diones (IIa-f) were synthesized from Embelin by treating alkyl and aryl halides in dichloromethane, sodium hydroxide and tetrabutyl ammonium bromide to give respective ethers. The title compounds evaluated for antimitotic activity, using germinating Bengal gram seeds and germinating Onions, *Allium cepa*. New Embelin derivatives demonstrated antimitotic activity in the order of IIf, IIa, IIb, IIe, IId, IIc. Among all the derivatives of Embelin, benzyl derivative (IIf) has shown significant activity when compared to rest of the compounds.

Key Words: Antimitotic activity, Embelin

Introduction

Embelin(2,5-dihydroxy-3-undecyl-1,4-benzoquinone), isolated from the berries of the plant *Embelia ribes,* is a species in the *Myrsinaceae*, it has been reported to possess anti-fertility (Radhakrishnan N *et al*., 1975) and antiimplantation (Krishnaswamy M *et al*., 1980) properties. In our earlier studies, the antitumour, antiinflammatory, analgesic and antioxidant (Chitra M *et al*., 1994) activities of Embelin were investigated. In this study, the effect of Embelin on lipid profile in cancer has been assessed as alterations in lipid levels might serve as indicators of tumour regression or progression. it has been shown to decrease testosterone levels (Githui *et al*., 1991), induce apoptosis in human myeloid HL-60 cells by targeting microtubular proteins (Xu *et al*., 2005) and induce cleavage of receptor-interacting protein (RIP) through activation of caspases during pancreatitis (Mareninova *et al*., 2006). In addition, it is effective against *N*-nitrosodiethylamine/phenobarbital-induced hepatocarcinogenesis in Wistar rats (Sreepriya and Bali, 2005). In view of these, a project was undertaken to synthesize a new series of 2-hydroxy-5-substituted-3-undecylcyclohexa-2,5-diene-1,4-diones and to evaluate new compounds for their antimitotic activity. Synthesis of title compounds was shown in Scheme 1.

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Materials and Methods

Melting points were determined in open capillary tubes, using Toshniwal melting point apparatus and are uncorrected. IR spectra were recorded on Perkin – Elmer spectrum BX-I series, FT IR spectrophotometer using KBr discs. PMR spectra were recorded on Brucker spectrospin 400 MHz spectrophotometer using TMS as an internal standard. Purity was checked by TLC using TLC aluminum sheets silica gel 60, supplied by E.Merk,

Mumbai, India. The physical constants, yield and analytical data of 2-hydroxy-5-substituted-3-undecylcyclohexa-2,5-diene-1,4-dione IIa–f are given in table 1.

Extraction and Isolation of Embelin:

The berries of *embelia ribes* (2 kg) were purchased from the local market. They were dried, size reduced and passed through 20 mesh. 2 kg of powdered *embelia berries* were soaked in n-hexane for 7 days. The level of n-hexane was maintained 1 inch above powdered *emdelica berries*. The contents were then referred for 2 hours. It is filtered at the vaccum pump while it is hot, upon cooling Embelin precipitates out, which was filtered and filtrate was again used to soak the material for second extraction for another week, the process is repeated.
The crude Embelin obtained from the extraction was crystallized from benzene. The filtrate of second extraction was concentrated and the product was also recrystalized from Benzene. The recrystalization process was repeated until golden crystals of Embelin (32g) were observed m.p. 142-144^0 C.

Reaction of Embelin with Alkyl halides Under "PTC (phase transfer catalyst)" conditions:
General Procedure:

The Embelin-5-O-Alkyl Ethers were prepared by vigorously stirring a mixture of Embelin (0.297 gms, 0.001 mole), aqueous sodium hydroxide (1.5 times the Embelin), alkylating agent (excess 2-3 times of the Embelin) and Tetrabutyl Ammonium bromide (catalytic amount) in Dichloromethane (DCM) for 3-20 hr at room temperature. The organic layer is separated, washed with Ammonia solution (2%) and then with sodium hydroxide solution (2N), saturated sodium chloride, dried and solvent was evaporated to get oily product. The product displayed two spots on TLC, it was chromato graphed over oxalic acid coated silicagel using solvents of increasing polarity from hexane through ethyl estate. The fractions were monitored through TLC.

After performing column chromatography orange coloured oily compounds were obtained as a product. This did not solidify even on standing at the ordinary temperature for a month. But on cooling in a refrigerator for a long time the compounds were become as semi solid mass (Gupta OP *et al*., 2006).

2,5-dihydroxy-3-undecylcyclohexa-2,5-diene-1,4-dione (I)

IR (KBr) (cm $^{-1}$): 1619.98(C=O), 3320.15 (OH), 3336.09(OH),1170 (C-O-C). ^{1} H-NMR ($CDCl_3$, 400 MHz), δ (ppm): 0.8-1.0 (t, 3H, CH_3), 1.5 (S, 18H,$(CH_2)_9$), 2.1-2.3 (t, 2H,CH_2(allylic)), 6.0 (S, 2H,2 OH), 7.3 (S,1H,Ar-H). LC-MS (m/z): 279.06 (M+1).

2-hydroxy-5-methoxy-3-undecylcyclohexa-2,5-diene-1,4-dione (II a)

IR (KBr) (cm $^{-1}$): 1615.83 (C=O), 3310.25 (OH), 1195.94 (C-O-C). ^{1} H-NMR ($CDCl_3$, 400 Hz), δ (ppm): 0.8-1.0 (t, 3H, CH_3), 1.5 (S, 18H,$(CH_2)_9$), 2.1-2.3 (t, 2H, CH_2(allylic)), 6.0 (S,H,OH), 7.3 (S, 1H, Ar-H) 3.4-3.5 (t, 3H, CH_3). LC-MS (m/z): 305.11 (M+1).

5-ethoxy-2-hydroxy-3-undecylcyclohexa-2,5-diene-1,4-dione(II b)

IR (KBr) (cm $^{-1}$): 1619.98(C=O), 3320.15 (OH), 1170 (C-O-C). ^{1} H-NMR ($CDCl_3$, 400 MHz), δ (ppm): 3.3-4.5 (q, 2H, OCH2), 0.8-0.9 (t, 3H, CH_3), 1.2-1.3 (t, 3H, CH_3), 1.5 (S, 18H,$(CH_2)_9$), 2.1-2.3 (t, 2H, CH_2 (allylic)), 6.0 (S, H, OH), 7.3 (S, 1H, Ar-H). LC-MS (m/z): 326.13 (M+1).

2-hydroxy-5-propoxy-3-undecylcyclohexa-2,5-diene-1,4-dione (II c)

IR (KBr) (cm $^{-1}$): 1661.32 (C=O), 3319.98 (OH), 1221.15 (C-O-C). ^{1} H-NMR ($CDCl_3$, 400 MHz), δ (ppm): . 3.2-3.4 (t, 2H, OCH2), 1.60-1.7 (m, 2H, CH2), 0.9-1 (t,3H,CH3), 0.8-1.0 (t, 3H, CH_3), 1.5 (S, 18H,$(CH_2)_9$), 2.1 - 2.3 (t, 2 H, CH_2 (allylic)), 6.0 (S, H, OH), 7.3 (S, 1H, Ar-H). LC-MS (m/z): 339.83 (M+1).

5-butoxy-2- hydroxy-3-undecyl benzo-1,4-quinone (II d)

IR (KBr) (cm $^{-1}$): 1658(C=O), 3320.17 (OH), 1200 (C-O-C). ^{1} H-NMR ($CDCl_3$, 400 MHz), δ (ppm): 3.3-3.4 (t,2H, OCH2), 1.60-.1.71(m, 4H, $2CH_2$), 0.86 - 0.92 (t, 3H, CH_3), 1.5 (S, 18H,$(CH_2)_9$), 2.1- 2.3 (t, 2H, CH_2 (allylic)), 6.0 (S, H, OH), 7.3 (S, H, Ar-H). LC-MS (m/z): 354.16 (M+1).

5-allyloxy-2- hydroxy-3-undecyl benzo-1,4-quinone (II e)

IR (KBr) (cm $^{-1}$): 1670(C=O), 3402 (OH), 1150(C-O-C). ^{1} H-NMR ($CDCl_3$, 400 MHz), δ (ppm): 5.9-6.0 (m, H, CH=), 3.4-3.6 (d, 2H, OCH_2), 5.2-5.3 (t, 2H,=CH_2),1.5 (S, 18H, $(CH_2)_9$), 2.1-2.3 (t,2H, CH_2 (allylic)), 6.0 (S,H,OH),7.3 (S,1H, Ar-H). LC-MS (m/z): 338.10 (M+1).

5-benzyloxy-2- hydroxy-3-undecyl benzo-1,4-quinone (II f)

IR (KBr) (cm^{-1}): 1713(C=O), 3390(OH), 1170(C-O-C). ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm): 3.2 (s, 2H, OCH_2), 1.5 (S, 18H,$(CH_2)_9$), 2.1 - 2.3 (t,2H,CH_2 (allylic)),6.0(S,H,OH),7.2-7.4(m,6H,Ar-H). LC-MS (m/z): 388.10 (M+1).

Anti mitotic activity:

1) Using germinating Bengal gram seeds

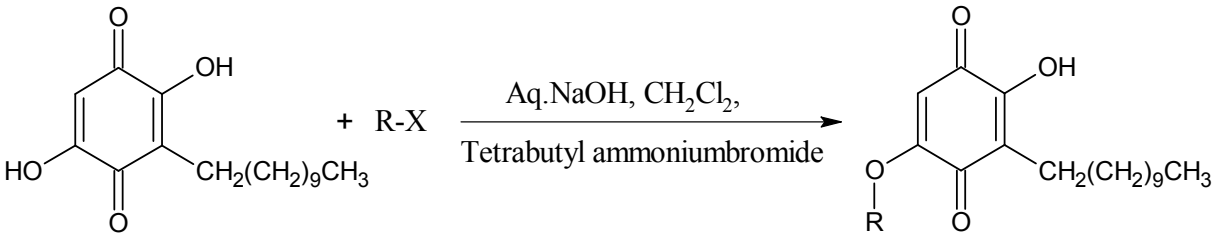
Bengal gram seeds of a good quality were taken and soken overnight with water to hasten the germination process. The next day the seeds were distributed in a group of 10 each in Petri dishes on moistened filter paper. Drug solutions were prepared 1% DMSO at concentrations ranging from 1ml and added to the filter paper in the Petri dishes. One Petri dish served as DMSO control, and one served as paclitaxol (positive) control. The seeds were allowed to germinate for 7 days and care was taken to moisten the filter paper with control and drug solutions every 24 hours. The length of the radicals was measured in cm at the end of 7th day and percent mean values of the DMSO (control) treated and % growth inhibition is calculated. The values are plotted on a graph to get IC_{50} value.

Antimitotic activity of the new derivatives of the Embelin showing good cytotoxic activity was done on germinating Bengal gram seeds. Embelin derivatives I-I_f tested for activity. Paclitaxel 10µg/ml was taken as positive control. The compounds were solubilized in solvents. Solvent was taken as control. The values of percentage inhibition of growth at various concentrations are given in the Table-2 and comparative percentage growth inhibition is shown in Figure-1.

2) Using germinating Onions, *Allium cepa*

Onions (*Allium cepa)* of a good quality were taken and hasten the germination process. The next day the bulbs were distributed in a group of two each in Petri dishes on moistened filter paper. New embelin derivatives were prepared in 1% DMSO at concentrations ranging from 1ml and added to the filter paper in the Petri dishes. One Petri dish served as solvent control, and one served as paclitaxel (positive) control. The bulbs were allowed to germinate for 7 days and care was taken to moisten the filter paper with control and extracts every 24 hours. The length of the radicals was measured in cm at the end of 7th day and percentage mean values of the control treated and percentage inhibition is growth is calculated. The values are plotted on a graph and IC_{50} values are calculated.

Antimitotic activity of the new derivatives of the Embelin showing good cytotoxic activity was done on germinating Bengal gram seeds. Embelin derivatives IIa to IIf tested for activity. Paclitaxel 10µg/ml was taken as positive control. The compounds were solubilized in solvents. Solvent was taken as control. The values of percentage inhibition of growth at various concentrations are given in the Table 3 and comparative percentage growth inhibition is shown in Figure-2.



I
Embelin

IIa-f

Scheme 1: Synthesis of 2-hydroxy-5-substituted-3-undecylcyclohexa-2,5-diene-1,4-diones

Table 1: Physical data of 2-hydroxy-5-substituted-3-undecylcyclohexa-2,5-diene-1,4-diones

Compound	R	Mol. Formula	Melting Point (^{0}C)	R_f	Yield (%)
I	H	$C_{17}H_{26}O_4$	142-144	0.51	60
IIa	$-CH_3$	$C_{18}H_{28}O_4$	165-168	0.52	72
IIb	$-CH_2-CH_3$	$C_{19}H_{30}O_4$	172-176	0.58	68
IIc	$-CH_2-CH_2-CH_3$	$C_{20}H_{32}O_4$	128-132	0.63	74
IId	$-CH_2-CH_2-CH_2-CH_3$	$C_{21}H_{34}O_4$	126-128	0.66	78
IIe	$-CH_2-CH = CH_2$	$C_{20}H_{30}O_4$	169-173	0.64	66
IIf	$-CH_2-C_6H_5$	$C_{24}H_{32}O_4$	112-116	0.68	58

Table 2: Comparative percentage growth inhibition of Bengal grams by Embelin derivatives.

Con (µg/ml)	Embelin(I)	I_a	I_b	I_c	I_d	I_e	I_f	Paclitaxel (Std)
125	20.2	25	25.3	36	35	50	65	100
250	28	31.3	31.4	39	40	87	93	100
500	36.1	48	47	50	43	91	95	100
1000	38	50	48	52	45	92	95	100

Table 3: Comparative percentage growth inhibition of *Allium cepa* using new embelin derivatives.

Con (µg/ml)	Embelin(I)	I_a	I_b	I_c	I_d	I_e	I_f	Paclitaxel (Std)
125	22.11	28.84	30.04	30.07	33.9	50.6	60.4	100
250	25.78	31.17	34.17	37.15	45.1	80.1	86	100
500	34.08	37.99	38.77	40	42.8	85	89.02	100
1000	36.7	40	40	42	43.6	86	90.03	100

Figure 1: Graph showing comparative percentage growth inhibition Bengal grams by using Embelin derivatives

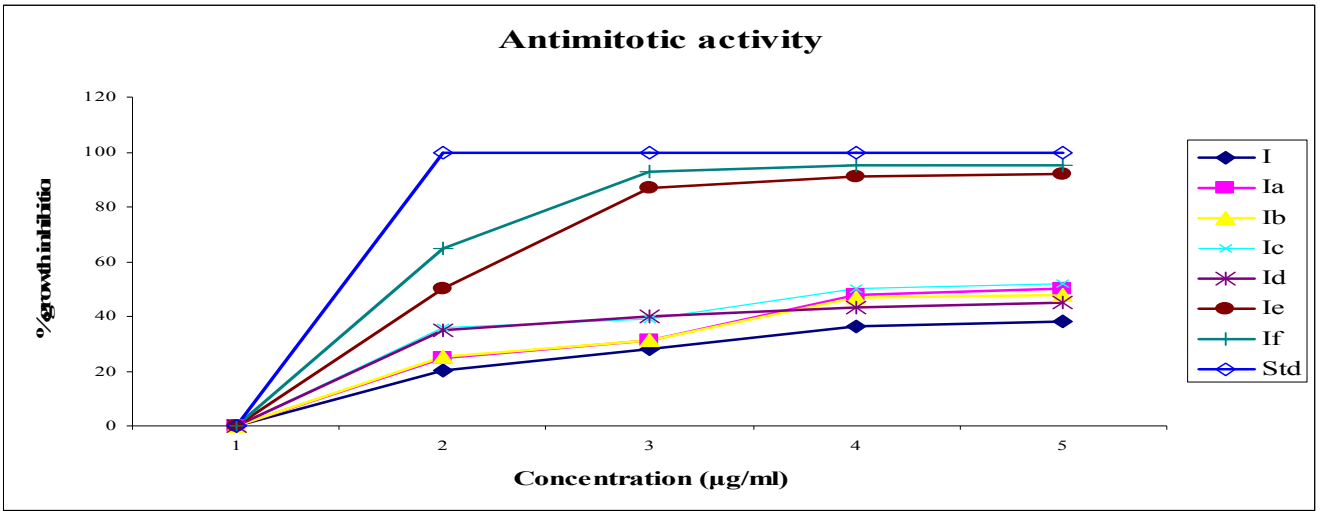
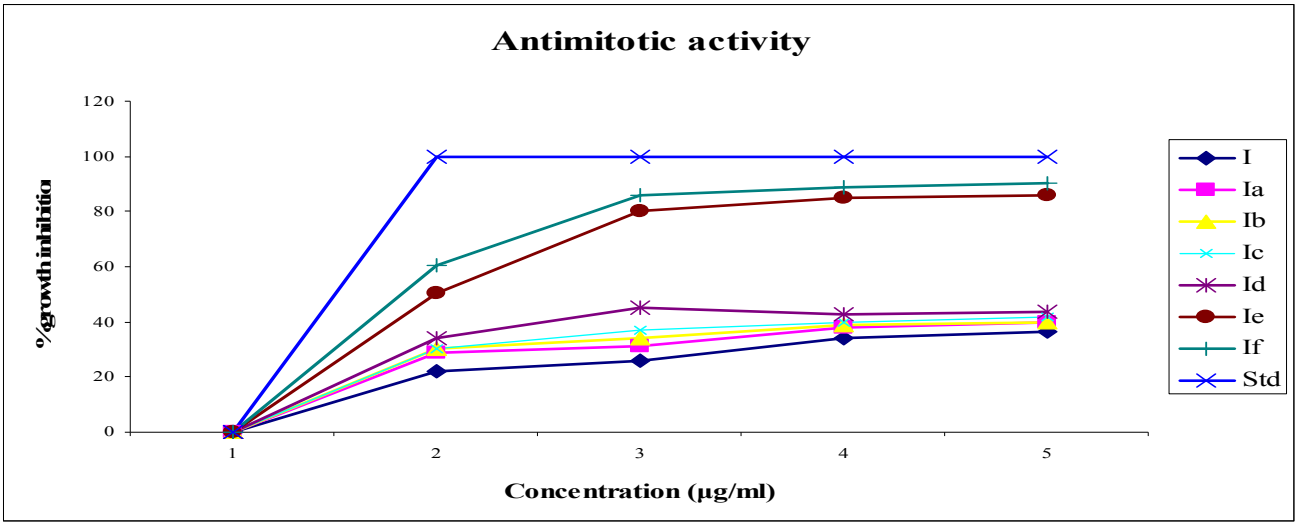


Figure 2: Graph showing comparative percentage growth inhibition *allium cepa* by using embelin derivatives.



Results and Discussion

All the new Embelin derivatives were synthesized according to the standard PTC procedure, as mentioned in the scheme by using Embelin as starting material. All the cases completion of the reactions were confirmed by thin layer chromatography (Ethylacetate : Hexane, 3:7) and characterized with the help of spectral data (IR, NMR) and the derivatives were purified by column chromatography with oxalic acid coated silica gel (60-120 mesh). All the synthesized derivatives were screened for antimititoc activity.

New Embelin derivatives demonstrated antimitotic activity in the order of IIf, IIa, IIb, IIe, IId, IIc. Among all the derivatives of embelin, benzyl derivative (IIf) has shown significant activity when compared to rest of the compounds.

Conclusion

In the present study, all the proposed Embelin (IIa, IIb, IIc, IId, IIe, IIf) derivatives were synthesized and their Antimitotic and wound healing activity was determined. Compound I_f demonstrated good Antimitotic Activity when compared to that of respective parent compound and the control.

Acknowledgments

The authors are thankful to the Director and Principal, Sri Rajya Laxmi (SRL) Institute of Pharmaceutical Sciences, Warangal, Kakatiya University and IICT- Hyderabad, for providing laboratory facilities and financial support.

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