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Synthesis and Evaluation of Analgesic and Anti-inflammatory Activities of Most Active Antioxidant Derivatives of Embelin

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Authors' contributions

This work was carried out in collaboration between all authors. Author SM designed the study, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Authors SB, SR, BST and VPV managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To evaluate antioxidant, analgesic and anti-inflammatory properties of embelin and its derivatives.
Methods: In the present study embelin was condensed with various aliphatic substituted primary amines, hydrazines and amino acids to yield seven new and five reported derivatives. All these compounds along with embelin were evaluated for *in vitro*

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antioxidant activity using ABTS and DPPH methods. Potent compounds were selected for *in vivo* analgesic and anti-inflammatory activities.
Results: Hydrazines, amino acids substituted embelin derivatives and phenazines showed potent antioxidant activity. These compounds along with embelin were studied for analgesic and anti-inflammatory activities at 10 and 20 mg/kg doses by standard methods. Potent analgesic activity higher than the standard pentazocine was observed. Embelin and its derivatives almost completely abolished the acetic acid induced writhing. Phenyl alanine and phenazine derivative showed better anti-inflammatory activity than embelin.
Conclusion: Further research would be of interest to explain the exact mechanism of these compounds and chemical modifications, biological screening and toxicity studies can also be explored.

Keywords: Embelin; structure activity relationship; antioxidant; analgesic; anti-inflammatory.

1. INTRODUCTION

Embelin(2,5-dihydroxy-3-undecyl-1,4-benzoquinone) is a naturally occurring alkyl substituted hydroxy benzoquinone and a major constituent of *Embelia ribes* Burm. (Family: Myrsinaceae). The plant is indicated in traditional medicine for the treatment of various diseases. The fruit is bitter in taste, good appetizer, cures tumors, ascites, bronchitis, jaundice, brain tonic, mental disorders, dyspnoea, diseases of the heart, urinary discharges, scorpion-sting, snake-bite and tooth ache [1]. It has been reported to posesess antioxidant properties in diabetic animals and anti-inflammatory to relive rheumatism and fever [2,3]. Embelin showed antifertility [4], anti-implantation [5], antitumour, anti-inflammatory, analgesic [6], antioxidant [7], hepatoprotective [8], wound healing [9], antibacterial [10] and anticonvulsant activities [11]. Quinonic compounds are ubiquitous in nature. They are implicated in numerous cellular functions and are involved in mechanisms of electron and hydrogen transfers. Quinones form a large class of antitumor agents approved for clinical use, and many other antitumor quinones are in different stages of clinical and preclinical development [12]. The efficiency of the quinonic compounds in inhibiting cancer cell growth is believed to stem from their participation in key cellular redox mechanisms with consequent generation of highly reactive oxygen species (ROS). The ROS turn out to modify and degrade nucleic acids and proteins within the cells [13]. One of the most simple 1,4-benzoquinonic compound isolated from natural sources is embelin. Padmanabha Rao and Venkateswarlu [14], reported a condensation reaction of embelin and various primary amines to afford di-imines. Gupta et al. [15], reported analgesic and anti-inflammatory properties of embelin di-imine and di-salt derivatives. It represents a promising lead compound for designing a new class of analgesic and anti-inflammatory agents. These antecedents justify the interest in the construction of newer embelin derivatives by using primary amines.

Free radicals play important roles in many physiological and pathological conditions [16]. In general, excess of free radicals caused by the imbalance between free radicals generation and scavenging may contribute to disease development. Painful stimulation increases the production of free radicals with increased lipoperoxidation. The application of antioxidants increases the antioxidative capacity and thus enhances the protection against the consequences of pain. Antioxidants are known to protect CNS against free radicals and also decrease the sensation of pain [16]. The role of reactive oxygen species in the

pathophysiology of inflammation is well-established. Free radicals can damage membranes, proteins, enzymes and DNA, increasing the risk of diseases such as cancer, Alzheimer's, Parkinson's, angiocardiopathy, arthritis, asthma, diabetes, and degenerative eye diseases [17]. Natural products, natural products derivatives, synthetic compounds with natural products-derived pharmacophore and synthetic compounds designed from natural products are also important to manage pathological conditions of those diseases caused by free radicals [17].

Due to the importance of embelin derivatives considerable efforts have been made by several investigators, to prepare new compounds bearing single substituent or more complicated systems, including amino acids rings mainly at 5-positions. Also, our earlier studies and literature survey reveals an excellent antioxidant, analgesic and antiinflammatory activity with some derivatives of embelin [18]. Encouraged by these findings we thought of preparing new derivatives to screen them for for *in vitro* antioxidant activity using standard ABTS and DPPH radical scavenging methods was carried out. The potent compounds were subjected to *in vivo* analgesic and anti-inflammatory screening in experimental animals.

2. MATERIALS AND METHODS

2.1 General

IR spectrum was recorded usingFT-IR, Perkin Elmer 8400 series instrument. NMR spectrum was obtained on a DDR X - 400 MHz and 100 MHz Bruker Daltonics, Germany. Absorbance was recorded by using Elisa Reader, Bio-Rad Laboratories Inc, California, USA, model 550. Mass spectrum was recorded by using Shimadzu MS-2010 A, Koyoto, Japan. Melting points (uncorrected) were obtained on a melting point apparatus, Lab India, Mumbai.

2,2′-diphenyl-1-picryl hydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) and carrageenan were obtained from Sigma Aldrich Co, St Louis, USA. Pentazocine was obtained from Ranbaxy, New Delhi, India. Diclofenac sodium was obtained from Wochardt Ltd, Mumbai, India. Rutin was obtained from Acros Organics, New Jersy, USA. Ascorbic acid was obtained from S.D. Fine Chem, Ltd., Biosar, India. All other chemicals used were of analytical grade.

2.2 Plant Material

The berries of *Embelia ribes* were purchased from Abirami Botanicals, Tuticorin, Tamilnadu, India and authenticated by Medicinal Plants Survey and Collection Unit, Ootacamund, Tamil Nadu, India, where a voucher has been deposited for further reference.

2.3 Extraction and Isolation of Embelin (1)

Coarsely powdered berries of *Embelia ribes* (2 kg) were exhaustively extracted with *n*-hexane by cold extraction method (3 x 2 l). After 72 h, the extracts were concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50℃). The residue so obtained was subjected to column chromatography over silica gel (100-200 mesh) and elution with benzene yielded an orange coloured powder [19], which on crystallization with ether afforded orange plates of embelin1 (2,5-dihydroxy-3-undecyl-1,4-benzoquinone, yield 6.5 g, 0.325%). It was found to be homogenous by HPTLC when

separated using the solvent system ethyl acetate: benzene (70:30). It was characterized by comparing its melting point, IR, NMR and MS data with literature values [20], mp 141-143℃; IR υ_{max}(KBr) cm^{-1}: 3308 (O-H), 2920, 2848 (C-H), 1643 (α, β- unsaturated C=O), 1614 (C=C), 1329, 1193; ^{1}H NMR (400 MHz, $CDCl_3$) δ : 7.68 (s, 2H, -OH), 6.00 (s, 1H, H-6), 2.44 (t, 2H, H-1'), 1.47 (m, 2H, H-2'), 1.25-1.30 (m, 16H, H-3' to 10'), 0.88 (t, 3H, H-11'); ^{13}C NMR (100 MHz, $CDCl_3$) see (Table 1); negative ESI-MS: m/z calculated for 294.18, Found: 293.

2.4 Condensation of Embelin with Primary Amines

2.4.1 General procedure

Embelin (1 mmol) and the primary amine (1 mmol) were boiled under reflux on a water bath at 100℃ for 2-3 h [14,15]. The cooled reaction mix ture was decomposed using excess of ice cold dil. HCl and the product obtained, was subjected to column chromatography over silica gel (100-200 mesh). The elution with petroleum ether and ethyl acetate (90:10) yielded the products.

2.5 5-(Methylamino)-2-Hydroxy-3-Undecylcyclohexa-2, 5-Diene-1, 4-Dione (2)

Obtained as pink rectangular prisms, mp 138-140℃; 0.271 g, yield 88.27%; IR v_{max} (KBr) cm^{-1}: 3309 (O-H), 3269 (N-H), 2918, 2850 (C-H), 1651 (α, β- unsaturated C=O), 1577, 1514, 1213 (C-N), 1145 (C-O), 790, 688; ^{1}H NMR (500 MHz, DMSO) δ: 10.49 (s, 1H, -NH), 7.80 (s, 1H, -OH), 5.30 (s, 1H, H-6), 2.73 (s, 3H, 1''-CH_3), 2.49 (t, 2H, H-1'), 1.48 (m, 2H, H-2'), 1.21-1.32 (m, 16H, H-3' to 10'), 0.84 (t, 3H, H-11'); ^{13}C NMR (100 MHz, DMSO) see (Table 1); negative ESI-MS: m/z calculated for 307.21, Found: 306 $[M-H]^{-1}$.

2.6 5-(Ethylamino)-2-Hydroxy-3-Undecylcyclohexa-2, 5-Diene-1, 4-Dione (3)

Obtained as pink solid, mp 132-134℃; yield 0.296 g , 92.21%; IR v_{max} (KBr) cm^{-1}: 3309 (O-H), 3277 (N-H), 2922, 2850 (C-H), 1641 (α, β- unsaturated C=O), 1568, 1504, 1213 (C-N), 1143 (C-O), 763, 740; ^{1}H NMR (500 MHz, DMSO) δ: 10.50 (s, 1H, -NH), 7.68 (s, 1H, -OH), 5.26 (s, 1H, H-6), 3.14 (t, 2H, H-1''), 2.24 (t, 2H, H-1'), 1.32 (m, 2H, H-2'), 1.09 (t, 3H, H-2''), 1.12-1.22 (m, 16H, H-3' to 10'), 0.84 (t, 3H, H-11'); ^{13}C NMR (100 MHz, DMSO) see (Table 1); negative ESI-MS: m/z calculated for 321.23, Found: 320 $[M-H]^{-1}$.

2.7 5-(Butylamino)-2-Hydroxy-3-Undecylcyclohexa-2, 5-Diene-1, 4-Dione (4)

Obtained as pink prisms, mp 145-147℃; yield 0.275 g, 78.80%; IR v_{max} (KBr) cm^{-1}: 3311 (O-H), 3273 (N-H), 2922, 2848 (C-H), 1641 (α, β- unsaturated C=O), 1570, 1508, 1211 (C-N), 1145 (C-O), 767, 704; ^{1}H NMR (500 MHz, DMSO) δ: 10.50 (s, 1H, -NH), 7.70 (s, 1H, -OH), 5.26 (s, 1H, H-6), 3.33 (t, 2H, H-1''), 3.10 (t, 2H, H-2''), 2.24 (t, 2H, H-1'), 1.48 (m, 2H, H-2'), 1.32 (t, 2H, H-3''), 1.22-1.31 (m, 16H, H-3' to 10'), 0.87 (t, 3H, H-4''), 0.89 (t, 3H, H-11'); ^{13}C NMR (100 MHz, DMSO) see (Table 1); negative ESI-MS: m/z calculated for 349.26, Found: 348 $[M-H]^{-1}$.

2.8 5-(4-Acetylphenylamino)-2-Hydroxy-3-Undecylcyclohexa-2, 5-Diene-1, 4-Dione (5)

Obtained as dark brown solid, mp 190-192℃; yield 0 .350 g, 85.16%; IR v_{max} (KBr) cm^{-1}: 3308 (O-H), 3238 (N-H), 2920, 2848 (C-H), 1681 (α, β- unsaturated C=O), 1637 (C=O), 1573, 1518, 1220 (C-N), 1176 (C-O), 767, 707; ^{1}H NMR (500 MHz, DMSO) δ: 10.80 (s, 1H, -NH), 9.40 (s, 1H, -OH), 7.98 (t, 2H, H-3'', 5''), 7.51 (d, 2H, H-2'', 6''), 5.97 (s, 1H, H-6), 2.55

(s, 3H, -$COCH_3$), 2.28 (t, 2H, H-1'), 1.39 (m, 2H, H-2'), 1.22-1.33 (m, 16H, H-3' to 10'), 0.84 (t, 3H, H-11'); ^{13}C NMR (100 MHz, DMSO) see (Table 1); negative ESI-MS: m/z calculated for 411.24, Found: 410 $[M-H]^{-1}$.

2.9 5-(4-((E)-3-Phenylacryloyl) Phenylamino)-2-Hydroxy-3-Undecylcyclohexa-2, 5-Diene-1, 4-Dione (6)

25 ml of the 10% NaOH and 25 ml of ethanol were taken in a 100 ml beaker and stirred continuously for 30 min. To this benzaldehyde (1 mmol) was added and stirred continuously for another half an hour, add compound 5 (1 mmol) at the last. The solution was allowed to stir for 3-4 h at room temperature. After completion of the reaction 100 ml of cold water was added, formed precipitate was filtered washed three times with 50 ml of water each time to remove sodium hydroxide. Then the precipitate was dried and re-crystallized from ethanol.

Obtained as black prisms, mp 182-184℃; yield 0.360 g, 72.14%; IR v_{max}(KBr) cm^{-1}: 3308 (O-H), 3238 (N-H), 2922, 2850 (C-H), 1660 (α, β- unsaturated C=O), 1597, 1514, 1479, 1219 (C-N), 1176 (C-O), 763, 692; ^{1}H NMR (500 MHz, DMSO) δ: 10.80 (s, 1H, -NH), 9.22 (s, 1H, -OH), 8.17-8.19 (m, 2H, H-11" and H-15"), 7.71 and 7.97 (m, 2H, CH=CH), 7.90 (d, 2H, H-2", 6"), 7.49 (d, 2H, H-3", 5"), 7.45-7.50 (m, 3H, H-12"-14"), 5.79 (s, 1H, H-6), 2.23 (t, 2H, H-1'), 1.30 (m, 2H, H-2'), 1.23 (m, 16H, H-3' to 10'), 0.84 (t, 3H, H-11'); ^{13}C NMR (100 MHz, DMSO) see (Table 1); negative ESI-MS: m/z calculated for 499.27, Found: 498 $[M-H]^{-1}$.

2.10 5-(O-Tolylamino)-2-Hydroxy-3-Undecylcyclohexa-2, 5-Diene-1,4-Dione (7)

Obtained as black prisms, mp 135-137ºC; yield 0.350, 91.38%; IR v_{max}(KBr) cm^{-1}: 3302 (O-H), 3259 (N-H), 2920, 2852 (C-H), 1637 (α, β- unsaturated C=O), 1566, 1508, 1213 (C-N), 1114 (C-O), 763, 717; ^{1}H NMR (500 MHz, DMSO) δ: 10.62 (s, 1H, -NH), 9.07 (s, 1H, -OH), 7.33 (m, 1H, H-6"), 7.25 (m, 2H, H-4", 5"), 7.17 (d, 1H, H-3"), 5.00 (s, 1H, H-6), 2.31 (s, 3H, 2"-CH_3), 2.15 (t, 2H, H-1'), 1.39 (m, 2H, H-2'), 1.23-1.27 (m, 16H, H-3' to 10'), 0.84 (t, 3H, H-11'); ^{13}C NMR (100 MHz, DMSO) see (Table 1); negative ESI-MS: m/z calculated for 383.25, Found: 382 $[M-H]^{-1}$.

2.11 4-(4-Hydroxy-3, 6-Dioxo-5-Undecylcyclohexa-1, 4-Dienylamino) Benzoic Acid (8)

Obtained as violet rectangular prisms, mp 170-172ºC; yield 0.371 g, 89.83%; IR v_{max}(KBr) cm^{-1}: 3309 (O-H), 3240 (N-H), 2920, 2848 (C-H), 1697 (C=O-OH), 1639 (α, β- unsaturated C=O), 1575, 1518, 1220, 1116, 767, 709; ^{1}H NMR (500 MHz, DMSO) δ: 10.86 (s, 1H,-COOH), 9.72 (s, 1H, -NH), 9.37 (s, 1H, -OH), 7.96 (d, 2H, H-2", 6"), 7.50 (d, 2H, H-3", 5"), 5.96 (s, 1H, H-6), 2.32 (t, 2H, H-1'), 1.40 (m, 2H, H-2'), 1.23-1.27 (m, 16H, H-3' to 10'), 0.86 (t, 3H, H-11'); ^{13}C NMR (100 MHz, DMSO) see (Table 1); negative ESI-MS: m/z calculated for 413.22, Found: 412 $[M-H]^{-1}$.

2.12 3-(4-Hydroxy-3, 6-Dioxo-5-Undecylcyclohexa-1, 4-Dienylamino)-3-Phenylpropanoic Acid (9)

Obtained as brown solid, mp 147-149ºC; yield 0.324 g, 73.47%; IR v_{max}(KBr) cm^{-1}: 3431, 3309 (O-H), 3234 (N-H), 2920, 2848 (C-H), 1618 (α, β- unsaturated C=O), 1585, 1465, 1220 (C-N), 1120 (C-O), 767, 688; ^{1}H NMR (500 MHz, DMSO) δ: 13.05 (s, 1H, -COOH), 10.50 (s, 1H, -NH), 9.25 (s, 1H, -OH), 7.20-7.50 (5H, aromatic), 5.77 (s, 1H, H-6), 5.36 (2H, -CH_2 of

phenylalanine), 2.49 (1H, -CH of phenylalanine), 2.30 (t, 2H, H-1'), 1.39 (m, 2H, H-2'), 1.22 (m, 16H, H-3' to 10'), 0.84 (t, 3H, H-11'); ^{13}C NMR (100 MHz, DMSO) see (Table 1); negative ESI-MS: m/z calculated for 441.25, Found: 395 $[M\text{-}COOH]^{-1}$.

2.13 5-(2-Phenylhydrazinyl)-2-Hydroxy-3-Undecylcyclohexa-2, 5-Diene-1, 4-Dione (10)

Obtained as brown prisms, mp 171-173ºC; yield 0.303 g, 78.91%; IR v_{max}(KBr) cm^{-1}: 3182 (O-H), 3063 (N-H), 2922, 2850 (C-H), 1621 (α, β- unsaturated C=O), 1599, 1581, 1496, 1298 (C-N), 1116 (C-O), 750, 690; ^{1}H NMR (500 MHz, DMSO) δ: 10.03 (s, 2H, -NH), 9.22 (1H, -OH), 7.19-7.50 (5H, aromatic), 5.69 (s, 1H, H-6), 2.32 (t, 2H, H-1'), 1.39 (m, 2H, H-2'), 1.22-1.32 (m, 16H, H-3' to 10'), 0.84 (t, 3H, H-11'); ^{13}C NMR (100 MHz, DMSO) see (Table 1); negative ESI-MS: m/z calculated for 384.24, Found: 383 $[M\text{-}H]^{-1}$.

2.14 5-(2-(2, 4-Dinitrophenyl) Hydrazinyl)-2-Hydroxy-3-Undecylcyclohexa-2, 5-Diene-1, 4-Dione (11)

Obtained as brown prisms, mp 162-164ºC; yield 0.385 g, 81.22%; IR v_{max}(KBr) cm^{-1}: 3317 (O-H), 3252, 3190 (N-H), 2922, 2850 (C-H), 1634 (α, β- unsaturated C=O), 1593 (C=C), 1465, 1330 ($-NO_2$), 1195 (C-N), 767, 649; ^{1}H NMR (500 MHz, DMSO) δ: 10.84 (s, 1H, NH), 10.65 (s, 1H, NH), 9.49 (s, 1H, -OH), 8.16-8.80 (3H, aromatic), 5.86 (s, 1H, H-6), 2.30 (t, 2H, H-1'), 1.35 (m, 2H, H-2'), 1.20-1.30 (m, 16H, H-3' to 10'), 0.90 (t, 3H, H-11'); ^{13}C NMR (100 MHz, DMSO) see (Table 1); negative ESI-MS: m/z calculated for 474.21, Found: 473 $[M\text{-}H]^{-1}$.

2.15 (S)-2-(4-Hydroxy-3, 6-Dioxo-5-Undecylcyclohexa-1, 4-Dienylamino)-3-(1H-Indol-3-Yl) Propanoic Acid (12)

Obtained as black powder, mp 152-154ºC; yield 0.328 g, 68.33%; IR v_{max}(KBr) cm^{-1}: 3309 (O-H), 3250 (-NH), 2920, 2848 (C-H), 1614 (α, β- unsaturated C=O), 1587 (C=C), 1195 (C-N), 1116 (C-O), 767, 690; ^{1}H NMR (500 MHz, DMSO) δ: 12.10 (s, 1H,-COOH), 8.93 (s, 2H, -NH), 8.31 (s, 1H, -OH), 6.80-7.25 (m, 4H, H-4'',5'',6'' and 7''), 5.77 (s, 1H, H-6), 5.37 (s, 1H, H-2''), 3.82 (s, 1H, H-10''), 2.70 (t, 2H, H-9''), 2.27 (t, 2H, H-1'), 1.51 (m, 2H, H-2'), 1.22-1.33 (m, 16H, H-3' to 10'), 0.83 (t, 3H, H-11'); ^{13}C NMR (100 MHz, DMSO) see (Table 1); negative ESI-MS: m/z calculated for 480.26, Found: 479 $[M\text{-}H]^{-1}$.

2.16 1-Undecylphenazine-2, 3-Diol (13)

Obtained as golden yellow prisms, mp 180-182ºC; yield 0.366 g, 90.44%; IR v_{max}(KBr) cm^{-1}: 3302 (O-H), 2918, 2850 (C-H), 1558 (C=C), 1215 (C-N), 1143, 1120 (C-O), 756, 594; ^{1}H NMR (500 MHz, DMSO) δ: 7.17 (s, 2H, H-2 and 3 -OH), 6.30 (s, 1H, H-4), 7.73-8.13 (aromatic H, 4H, H-5,6,7 and 8), 3.10 (t, 2H, H-1'), 1.60 (m, 2H, H-2'), 1.20-1.40 (m, 16H, H-3' to 10'), 0.90 (t, 3H, H-11'); ^{13}C NMR (100 MHz, DMSO) see (Table 1); negative ESI-MS: m/z calculated for 366.23, Found: 365 $[M\text{-}H]^{-1}$.

Table 1. ^{13}C NMR of embelin and its derivatives

Carbons	1	2	3	4	5	6	7	8	9	10	11	12	13
1	a)	182.41	182.52	a)	a)	a)	a)	183.18	a)	a)	a)	183.16	114.78
2	a)	156.81	156.74	a)	a)	a)	a)	167.18	a)	a)	a)	156.79	152.00
3	116.99	115.35	115.42	115.38	115.35	114.50	115.97	117.22	115.76	117.82	114.73	115.49	151.42
4	a)	178.18	178.29	176.05	a)	a)	a)	181.41	a)	a)	a)	181.40	120.20
5	a)	150.19	148.96	150.19	151.48	152.13	148.07	155.97	151.74	151.48	147.42	151.68	133.36
6	102.15	91.56	91.48	91.49	91.48	104.31	93.83	97.40	94.61	104.27	105.83	94.62	131.75
Chain 1' to	22.51 to	21.92 to	21.96 to	21.96 to	22.56	22.43 to	22.08 to	22.41 to	22.40 to	22.43 to	22.39 to	22.04 to	22.52 to
10'	31.90	31.27	31.27	31.26	to 31.76	31.73	31.27	31.74	31.74	31.73	31.75	31.75	31.71
11'	14.09	13.93	13.93	13.93	14.42	14.39	13.94	14.39	14.39	14.39	14.39	14.38	14.38
1''	-	31.27	36.77	41.70	151.50	152.13	135.98	142.73	129.62	142.89	142.24	121.47	-
2''	-	-	12.95	29.42	122.64	114.76	134.22	130.99	128.96	127.73	130.68	129.10	-
3''	-	-	-	19.64	130.05	130.64	126.81	122.72	129.21	127.53	127.56	130.60	-
4''	-	-	-	13.61	122.64	128.26	127.24	145.20	126.28	128.52	142.24	124.62	-
5''	-	-	-	-	130.05	129.89	126.43	117.83	128.70	127.66	126.99	127.07	-
6''	-	-	-	-	122.64	117.35	130.97	127.06	126.28	127.59	127.50	128.22	-
$-CH_3$	-	-	-	-	-	-	17.31	-	-	-	-	-	-
-COOH	-	-	-	-	-	-	-	197.01	192.00	-	-	-	-
$-CH_{2,}$ -CH	-	-	-	-	-	-	-	-	36.19, 56.12	-	-	-	-
7''or 7	-	-	-	-	177.44	172.46	-	-		-	-	125.71	130.80
8'' or 8	-	-	-	-	31.76	122.10	-	-	-	-	-	130.02	130.60
9'' or 9	-	-	-	-	-	130.85	-	-	-	-	-	29.99	147.44
10'' or 10	-	-	-	-	-	129.73	-	-	-	-	-	63.32	138.73
11'' or 11	-	-	-	-	-	127.49	-	-	-	-	-	179.31	138.90
12'' or 12	-	-	-	-	-	129.55	-	-	-	-	-	-	133.67
13''						126.78							
14''						129.55							
15''						126.78							

[a)]Carbon peaks not appeared due to fluxional effect

2.17 *In vitro* Antioxidant Activity

2.17.1 Preparation of test and standard solutions

Embelin (1), all the synthesized compounds (2-13), and the standard antioxidants, ascorbic acid and rutin were dissolved in distilled dimethyl sulphoxide (DMSO) separately and used for the *in vitro* antioxidant assays using ABTS and DPPH methods. The stock solutions were serially diluted with DMSO to obtain lower dilutions. Absorbance was measured against a blank solution containing the compounds or standards, but without the reagents. A control test was performed without the compounds or standards. The IC_{50} value, which is the concentration of the sample required to inhibit 50% of radical was calculated.

2.17.2 Scavenging of ABTS radical cation

Accurately 54.8 mg of ABTS was weighed and dissolved in 50 ml of distilled water (2 mM). Potassium persulphate (17 mM, 0.3 ml) was then added. The reaction mixture was left to stand at room temperature overnight in dark before usage. To 0.2 ml of various concentrations of the compounds 1-13 or standards, 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution were added to make the final volume to 1.36 ml. Absorbance was measured after 20 min spectrophotometrically at 734 nm [21].

2.17.3 DPPH radical scavenging method

A 10 µl aliquot of the different concentrations of the compounds (1-13) and standards were added to 200 µl of DPPH in methanol solution (100 µM) in a 96-well microtitre plate (Tarson Products (P) Ltd., Kolkota, India). After incubation at 37ºC for 20 min, the absorbance of each solution was determined at 490 nm [21] using ELISA reader (Bio-Rad Laboratories Inc., CA, USA, Model 550).

2.18 *In vivo* Analgesic and Anti-inflammatory Activities

2.18.1 Animals

The animals were obtained from the animal house of Sree Siddaganga College of Pharmacy, Tumkur, India, maintained under standard conditions (12 h light / dark cycle; 25±3ºC, 45-65% humidity) and had free access to standard rat feed and water *ad libitum*. All the animals were acclimatized to laboratory conditions for a week before commencement of the experiment. The experiments were performed during the light portion between 07:00-18:00 h to avoid circadian influences. Animal studies were performed according to the prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India.

2.18. 2 Analgesic activity

Three different sets of mice were randomized into eight groups, each containing six animals and used in three different models for the evaluation of analgesic activity. Different doses of compounds 1, 10 and 13 were prepared as suspensions in Tween-80 (1% v/v in saline). Two doses of embelin and its derivatives (10 and 20 mg/kg) were selected based on an earlier study [13].

Group I were treated with Tween-80 (1% v/v in saline) as normal vehicle control. Groups II-VII were treated with compounds 1, 10 and 13 at 10 and 20 mg/kg, respectively and Group VIII animals were treated with standard pentazocine at 20 mg/kg. All the treatments were administered intraperitoneally.

2.18.3 Eddy's hot-plate method

Mice were treated and placed on Eddy's hot plate kept at a temperature of 55±0.5ºC. A cut off period of 15 sec was observed to avoid damage to the paw. Reaction time and the type of response were noted using a stopwatch. The response is in the form of jumping, withdrawal of the paws or licking of the paws. The latency was recorded before and after 15, 30 and 45 min following the treatments. The percentage protection was calculated using the formula, protection (%) = (t-n/t) ×100, where, t = reaction time of treated group and n = reaction time of normal group [22].

2.18.4 Tail immersion method

In this method [23], 5 cm of the end of the mice tail was immersed in warm water maintained at 55±0.5ºC. The tail withdrawal reflex was recorded before and after 60 min following the treatments. The percentage protection was calculated as per hot plate method.

2.18.5 Acetic acid induced writhing method

In the acetic acid induced writhing [22] in mice an intraperitoneal injection of acetic acid (1%, 10 ml/kg) was given 30 min after the treatments. The response is in the form of abdominal contractions, trunk twist and extension of hind limb. The number of writhing in each mouse was counted for 20 min from the injection of acetic acid. The percentage protection was calculated using the formula, protection (%) = (c-t/c) ×100, where, t = reaction time of treated group and c = reaction time of control group.

2.19 Anti-inflammatory Activity

2.19.1 Carrageenan induced paw edema in rats

Swiss albino rats (150-200 g) were divided into eight groups with six animals in each group. Group I was served as control and received Tween-80 (1% v/v in saline). Groups II-VII were received the treatments as described in analgesic activity. Group VIII was treated as positive control and received standard diclofenac (20 mg/kg). All the treatments were administered intraperitoneally. The initial hind paw volume of rats was determined volumetrically by using a plethysmometer [24]. A solution of carrageenan in saline (1%, 0.1 ml/rat) was injected subcutaneously into the right hind paw 30 min after the treatments. The animals in the control group received the vehicle only. Paw volumes were measured up to 6 h at intervals of 30, 60, 120, 180 and 360 min and percent increase in edema between the control and treated groups were compared. The percentage protection was calculated as acetic acid induced writhing method.

2.20 Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by multiple comparison using the Dunnet's test. P values <0.05 were considered as significant.

3. RESULTS

3.1 Chemistry

The protocol for the synthesis of embelin derivatives is shown in (Fig. 1). Condensation of embelin with various primary amines, methylamine (2), ethylamine (3), butylamine (4), 4-aminoacetophenone (5), O-toluidine (7), 4-amino benzoicacid (8), phenylalanine (9), phenylhydrazine (10), 2,4-dinitro phenyl hydrazine (11), DL-tryptophan (12), O-phenylenediamine (13) and compound 5 reaction with benzaldehyde (6), gave seven new compounds and six known compounds.All these compounds were characterized by spectral studies (IR, ^{1}H NMR, ^{13}C NMR and mass) for the first time. The data were in accordance with the reported structures.

Embelin (1) was isolated from *Embelia ribes* using known procedure and characterized by comparison of its physical and spectral data with literature values [19] and with authentic sample available with us. The ^{13}C NMR spectrum of embelin did not exhibit all the ring carbon signals, but showed only two peaks at δ 116.99 (C-3) and 102.15 (C-6). Normally, the ^{13}C NMR of 2,5-dihydroxy-3alkyl-1,4-benzoquinones do not show the ring carbon peaks particularly those attached to oxygen atoms due to fluxional effect caused by intramolecular hydrogen bonding. The result of this is long spin relaxation time which leads to saturation of oxygen-carbon signals [20]. The fluxional effect can be precluded if at least one of the hydroxyl group is removed through structural modification. Such modifications lead to observation of all the ring carbons except compounds 4, 5, 6, 7, 9, 10 and 11 as shown in (Table 1 above). The IR, ^{1}H NMR and mass spectral data of these compounds confirmed their structures.

All the compounds (2-13, except 9) showed pseudomolecular ions for the $[M-H]^-$ ion in the mass spectra at m/z 306 (2), 320 (3), 348 (4), 410 (5), 498 (6), 382 (7), 412 (8), 383 (10), 473 (11) 479 (12) and 365 (13) respectively and $[M-COOH]^-$ peak at 395 (9), respectively. The IR spectra of compounds 2-12 showed absorptions between 3230 – 3400 cm^{-1} due to –OH and –NH groups and the absorption bands at 2840 to 2950 cm^{-1} are due to the presence of long aliphatic chain. The compounds also exhibited absorptions at 1635 to 1645 cm^{-1} due to the presence of α, β- unsaturated C=O and the peak present in between 1550 – 1620 cm^{-1} due to the presence of aromatic C=C. The absorption bands at 1213 and 1143 cm^{-1} were due to the C-N and C-O groups. Complementing these data, the reaction has taken place in the position C-5 and the -OH group of the embelin was substituted with –NH group.

In ^{1}H NMR spectrum of 2-12, -NH proton was observed at δ 8.93 – 10.84 and H-6 was observed at δ 5.00 to 6.00. The signals between δ 6.00 – 8.50 were assigned for aromatic protons and the rest of the signals between δ 0.80 – 2.50 were due to the presence of aliphatic chain. In ^{13}C NMR spectrum of 2-12, aromatic carbons appeared between δ 110.00 - 152.00, C-6 appeared between δ 91.00 – 106.00 and C-5 appeared at δ 146.00 to 152.00, confirms that the reaction has taken place at C-5 position. The reaction has occurred in only one position at C-5, but not in the C-2 position and this may be due to the steric factor.

The IR and NMR spectral data of compound 2 are almost identical with the parent compound 1. In addition a singlet signal at δ 2.73 for three protons in ^{1}H NMR and the corresponding signal at 31.27 in ^{13}C NMR for the presence of the N-methyl group. The compound 3 in addition to the embelin moiety signals it showed characteristic signals at δ 3.14 (t, 2H, H-1''), 1.09 (t, 3H, H-2'') showing the presence of N-ethyl group. It was supported

by the respective signals at δ 36.77 (C-1'') and 12.95 (C-2'') in the ^{13}C NMR spectrum. In compound 4 the butyl chain protons resonated at δ 3.33 (t, 3H, H-1''), 3.10 (t, 2H, H-2''), 1.32 (t, 2H, H-3'') and 0.87 (3H, t, H-4'') in the ^{1}H NMR and supported the signals at δ 41.70 (C-1''), 29.42 (C-2''), 19.64 (C-3'') and 13.61 (C-4'') in the ^{13}C NMR spectrum.

In compound 5, the singlet signal at δ 2.55 for three protons is due to the methyl group attached to the keto carbonyl group. It was supported by its ^{13}C NMR spectral signals by exhibiting a peak at δ 31.76 and 177.44, respectively. The compound 6, ^{1}H NMR spectrum in addition to the signals of compound 5 except the methyl signal at δ 2.55, it exhibited a pair of doublet signals at δ 7.71 (H-7''') and 7.97 (H-8''') each for one proton with a coupling constant of J=16 Hz indicating the presence of a trans alkene moiety. The signals at δ 8.17-8.19 (2H, m, H-11'', H-15''), 7.45-7.50 (3H, m, H-12''-H-14'') for the aromatic ring of benzaldehyde. In compound 7, the^{1}H NMR spectrum, the methyl group resonated at δ 2.31 as a singlet for three protons, corresponding to the peak at at δ 17.31 in the ^{13}C NMR. Compound 8, exhibited characteristic signal at δ 10.86 assigned for –COOH, corresponding to the peak at δ 197.01 in the ^{13}C NMR. Compound 9, ^{1}H NMR in addition to the signals of the embelin moiety it showed signals for two benzylic protons at δ 5.36 and one –CH group of phenylalanine peak appeared at δ 2.49, corresponding to the peak at δ 36.19 and 56.12 in ^{13}C NMR, respectively. The peak present at δ 13.05 is due to the presence of –COOH group. The aromatic protons resonated between δ 7.20 to 7.50 and the peak present at δ 192.00 for C=O in ^{13}C NMR spectrum.

In compound 10 formed reaction between embelin and phenyl hydrazine exhibited a two -NH protons, in its ^{1}H NMR spectrum resonated at δ 9.22 (s, 2H). Compound 11 in its IR spectrum exhibited characteristic IR bands at 1330 cm^{-1} for NO_2 groups, δ 9.29 (s, 2H) for two –NH group in the ^{1}H NMR. Compound 12, the^{1}H NMR exhibited signals for –COOH at δ 10.58, the signal at δ 3.82 for one proton is due to the –CHgroup attached to the –NH and –COOH group of tryptophan. The corresponding carbon signal appeared at δ 63.32 in the ^{13}C NMR. The peak present at δ 2.70 for two protons of –CH_2 group and corresponding to δ 29.99 in ^{13}C NMR.

The absence of carbonyl carbon signals in the ^{13}C NMR at δ 180-184 and in IR spectrum at 1635-1645 cm^{-1} suggested that the carbonyl groups of embelin have reacted with O-phenylenediamine to form the compound 13. The aromatic protons resonated at δ 7.17 (s, 1H, H-4), 8.05 (d, 1H, H-5), 7.75 (t, 1H, H-6), 7.80 (t, 1H, H-7) and at 8.13 (d, 1H, H-8). The respective carbon signals appeared at 114.78 to 152.00 (C-1 to C-12) in ^{13}C NMR.

3.2 *In vitro* Antioxidant Activity

Embelin showed potent antioxidant activity with IC_{50} values 0.23±0.04 µg/ml and 27.92±1.73 µg/ml in ABTS and DPPH methods [21], respectively. The phenylalanine (9), phenylhydrazine (10), 2,4-dinitro phenyl hydrazine (11), DL-tryptophan (12) and O-phenylenediamine (13) derivatives of embelin exhibited potent antioxidant activity better than embelin withIC_{50} values 0.19±0.02, 0.28±0.04, 0.21±0.02, 0.13±0.04 and 0.20±0.02 µg/ml in ABTS and 13.98±0.55, 24.88±1.25, 22.50±0.80, 14.70±0.59 and 13.60±0.91 µg/ml in DPPH methods, respectively. Potent antioxidant activity with very low IC_{50} values were obtained for all the compounds in ABTS method. The activity was found to be more than the standard ascorbic acid in all the compounds and more than standard rutin in compounds 1 and 8-13 (Table 2).

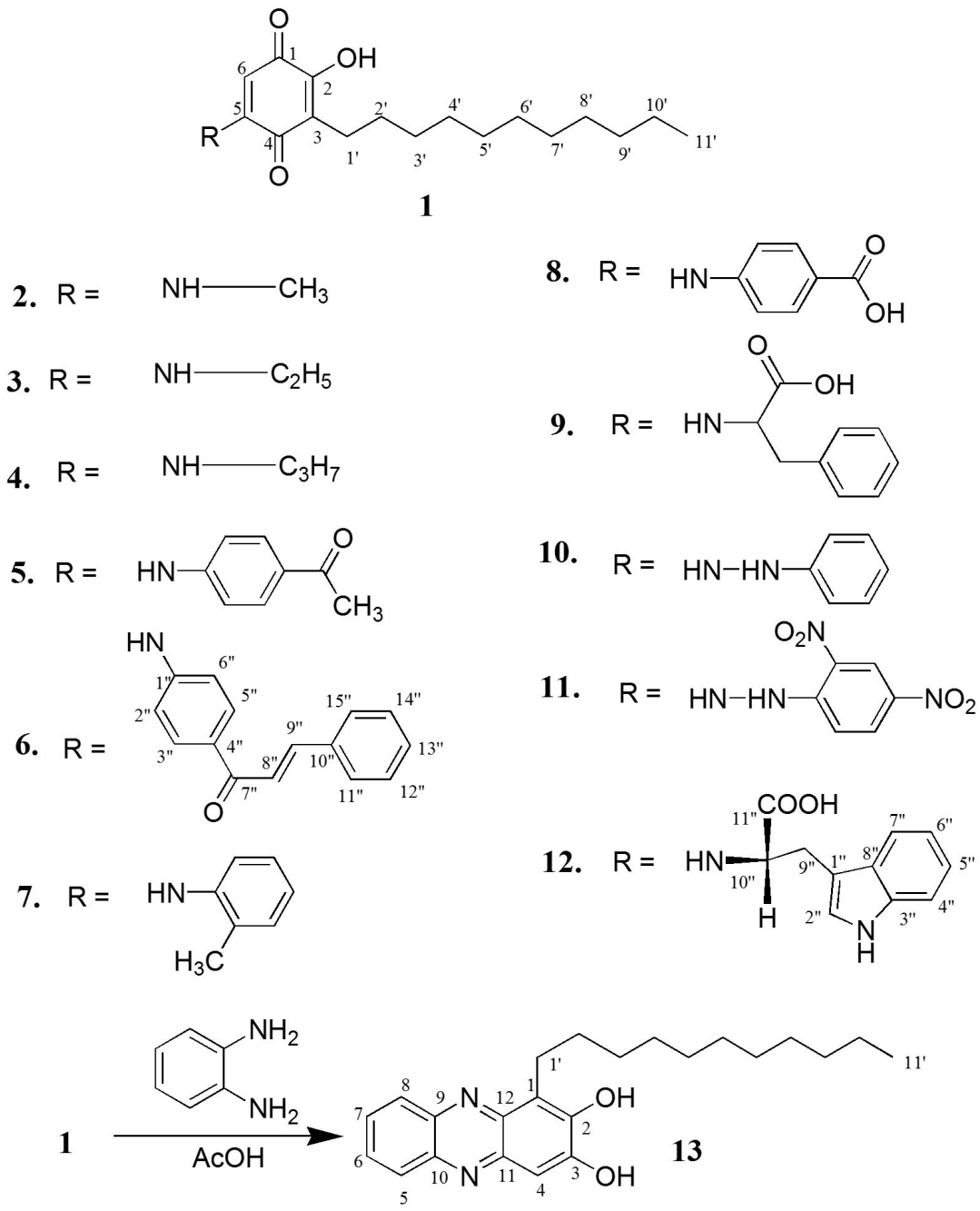

Fig. 1

Fig. 1. The structure of embelin and its synthetic derivatives

Table 2. *In vitro* antioxidant activity of embelin derivatives by using ABTS and DPPH methods

Compound	IC_{50} values ± SEM (µg/ml) by methods[a)]		Compound	IC_{50} values ± SEM (µg/ml) by methods[a)]	
	ABTS	DPPH		ABTS	DPPH
1	0.23±0.04	27.92±0.33	8	0.50±0.08	48.80±2.07
2	0.60±0.12	84.20±1.27	9	0.19±0.02	13.98±0.55
3	0.72±0.13	92.60±3.78	10	0.28±0.04	24.88±1.25
4	0.88±0.20	148.40±4.84	11	0.21±0.02	22.50±0.80
5	1.32±0.18	81.60±3.98	12	0.13±0.04	14.70±0.59
6	1.67±0.35	>250	13	0.20±0.02	13.60±0.91
7	0.78±0.11	73.80±4.74	Ascorbic acid	11.25±0.49	4.92±0.28
			Rutin	0.52±0.04	8.91±0.10

[a)] *Average of three determinations*

In DPPH method, all the compounds were found to possess higher IC_{50} values than standard ascorbic acid and rutin indicating the activity lesser than the standards. However, among all the compounds 1 and 9-13 were found to possess potent and compound 8 showed moderate antioxidant activities. Based on these results, compounds 9-13 along with 1 were chosen for comparing their *in vivo* analgesic and anti-inflammatory activities with embelin.

3.3 Analgesic Activity

In the hot plate method of analgesic activity [22], embelin its derivatives exhibited potent activity. The response time observed for the all the six compounds were significantly increased when compared to normal control (Table 3). The activity observed was found to be higher than the standard pentazocine after 15 and 30 min for compound 13 at 20 mg/kg. However, the standard pentazocine was found to be better active than all the three compounds during 45 min response. The percentage protection after 45 min for all the compounds ranged between 50.70 to 74.01%. Compounds 10, 12 and 13 were found to be better active than embelin.

In the tail immersion [23] and acetic acid induced writhing [22] methods, all the six compounds showed dose dependent and potent analgesic activity. The values were significant for all the compounds at both the doses in the acetic acid induced writhing and for compounds 1, 9, 11 and 13 at 20 mg/kg in tail immersion method (Table 4). The activity was found to be higher than the standard pentazocine for all the compounds in acetic acid induced writhing, but failed in tail immersion method. All the derived compounds 9, 10, 11, 12 and 13 were found to be more active than embelin at both the doses in tail immersion method and the activity was almost equivalent for all the six compounds in the acetic acid induced writhing method (Table 4). At higher dose, all the compounds almost completely abolished the writhing indicating their potent analgesic activity.

3.4 Anti-inflammatory Activity

Against carrageenan induced paw edema in rats [24], embelin given intraperitoneally at 20 mg/kg significantly reduced the paw edema after 120, 180 and 360 min when compared to control (Tables 5 and 6). Compounds 1, 10, 12 and 13 exhibited significant activity at 20

mg/kg after the 120 min and compound 9, 10, 11 and 13 at 20 mg/kg after 180 and 360 min. However, compounds 9 and 13 produced significant activity at 20 mg/kg dose during 30 to 360 min measurements. The standard diclofenac at 20 mg/kg also produced similar and better results than the tested samples.

Table 3. Analgesic activity of embelin and its derivatives by using hot plated method

Treatments (dose, mg/kg, i.p.)	Latency period, s (% Protection)		
	15 min	30 min	45 min
Normal	2.32±0.25	2.27±0.26	2.43±0.28
1 (10)	3.12±0.22 (25.64)	4.78±0.59 (52.51)	7.28±0.37** (66.62)
1 (20)	4.46±0.67* (47.98)	6.11±0.96* (62.85)	7.39±1.07** (67.12)
9 (10)	3.52±0.52 (34.09)	5.41±0.78 (58.04)	5.80±0.56 (58.10)
9 (20)	3.85±0.39 (39.74)	6.73±0.70** (66.27)	7.27±0.65** (66.57)
10 (10)	4.03±0.40 (42.43)	4.58±0.27 (50.44)	6.10±0.34 (60.16)
10 (20)	4.77±0.51* (51.51)	5.87±0.54* (61.33)	8.11±0.66*** (70.03)
11 (10)	3.66±0.41 (36.61)	5.05±0.53 (55.05)	5.49±0.57 (55.73)
11 (20)	3.97±0.41 (41.56)	5.34±0.72 (57.50)	6.34±0.77* (61.67)
12 (10)	4.18±0.49 (44.50)	4.58±0.53 (50.44)	4.93±0.61 (50.70)
12 (20)	4.75±0.63* (51.16)	6.16±0.86* (63.15)	8.11±0.87* (70.03)
13 (10)	4.95±0.48* (53.13)	5.42±0.72 (58.12)	7.08±1.06* (65.68)
13 (20)	6.01±0.57*** (61.40)	7.55±1.78*** (69.93)	9.35±1.53*** (74.01)
Pentazocine (20)	5.11±0.32** (54.60)	6.85±0.50** (66.86)	10.61±1.14*** (77.10)

*Values are given as mean ± S.E.M. for groups of six animals each, Dunnet's test; values are statistically significant at ***P<0.001, **P<0.01, *P<0.05 between control and treated groups.*

Table 4. Analgesic activity of embelin and its derivatives by using tail immersion and acetic acid induced writhing methods

Treatments (dose, mg/kg, i.p.)	Tail immersion		Treatments (dose, mg/kg, i.p.)	Acetic acid induced writhing	
	Latency period (s)	% Protection		No. of writhing	% Protection
Normal	4.39±0.60	-	Control	43.83±2.32	-
1 (10)	5.46±0.35	19.59	1 (10)	3.00±0.68***	93.15
1 (20)	7.84±0.69*	44.00	1 (20)	0.83±0.48***	98.11
9 (10)	6.49±0.98	32.35	9 (10)	10.33±1.82***	76.43
9 (20)	8.69±0.50**	49.48	9 (20)	1.83±0.65***	95.82
10 (10)	5.49±0.47	20.03	10 (10)	5.17±1.99***	88.20
10 (20)	7.52±0.68	41.62	10 (20)	1.33±0.67***	96.97
11 (10)	5.85±0.52	24.95	11 (10)	6.67±1.48***	84.78
11 (20)	8.83±0.41**	50.58	11 (20)	2.33±0.71***	94.68
12 (10)	5.81±0.96	24.44	12 (10)	8.67±1.74***	80.22
12 (20)	7.55±0.73	41.85	12 (20)	2.50±1.18***	94.30
13 (10)	6.17±0.39	28.85	13 (10)	2.50±0.85***	94.30
13 (20)	9.22±0.72***	52.39	13 (20)	0.67±0.33***	98.47
Pentazocine (20)	10.21±1.00***	57.00	Pentazocine (20)	7.67±1.45***	82.50

*Values are given as mean ± S.E.M. for groups of six animals each, Dunnet's test; values are statistically significant at ***P<0.001, **P<0.01, *P<0.05 between control and treated groups*

Table 5. Anti-inflammatory activity of embelin and its derivatives by carregeenan induced paw edema in rats

Treatments (dose, mg/kg, i.p.)	Paw volume, ml (% Protection)					
	0 min	30 min	60 min	120 min	180 min	360 min
Control	0.84±0.02	1.63±0.04	1.77±0.05	1.90±0.07	1.96±0.07	2.13±0.06
1 (10)	0.90±0.04	1.62±0.05(0.61)	1.73±0.04(2.26)	1.84±0.11(3.15)	1.82±0.03(7.14)	1.80±0.07*(15.49)
1 (20)	0.80±0.02	1.41±0.06(13.50)	1.54±0.07(12.99)	1.62±0.06*(14.74)	1.59±0.05**(18.88)	1.45±0.07***(31.92)
9 (10)	0.83±0.06	1.49±0.06(8.59)	1.56±0.07(11.86)	1.74±0.06(8.42)	1.69±0.04*(13.78)	1.57±0.07***(28.17)
9 (20)	0.95±0.07	1.34±0.06*(17.79)	1.48±0.05*(16.38)	1.65±0.05(13.16)	1.65±0.05**(15.82)	1.53±0.04***(26.29)
10 (10)	1.05±0.04	1.62±0.04(0.61)	1.75±0.04(0.11)	1.76±0.05(7.37)	1.68±0.09*(14.29)	1.61±0.09***(23.47)
10 (20)	0.95±0.04	1.54±0.07(5.52)	1.71±0.07(3.39)	1.64±0.07*(13.68)	1.60±0.04**(18.37)	1.51±0.02***(29.11)
Diclofenac (20)	0.89±0.04	1.22±0.05***(25.15)	1.25±0.05***(29.38)	1.30±0.03***(31.58)	1.16±0.02***(40.82)	1.13±0.03***(46.94)

Values are given as mean ± S.E.M. for groups of six animals each, Dunnet's test; values are statistically significant at ***P<0.001, **P<0.01, *P<0.05 between control and treated groups

Table 6. Anti-inflammatory activity of embelin and its derivatives by carregeenan induced paw edema in rats

Treatments (dose, mg/kg, i.p.)	Paw volume (ml)					
	0 min	30 min	60 min	120 min	180 min	360 min
Control	0.84±0.02	1.63±0.04	1.77±0.05	1.90±0.07	1.96±0.07	2.13±0.06
11 (10)	0.97±0.04	1.60±0.10(1.84)	1.67±0.09(5.65)	1.86±0.02(2.11)	1.79±0.04(8.67)	1.66±0.03***(24.41)
11 (20)	0.99±0.04	1.56±0.06(4.30)	1.61±0.06(9.04)	1.81±0.02(4.74)	1.73±0.03*(11.73)	1.61±0.04***(22.07)
12 (10)	0.91±0.05	1.62±0.06(0.06)	1.74±0.05(1.70)	1.78±0.04(6.32)	1.77±0.04(9.69)	1.69±0.04(20.67)
12 (20)	1.00±0.04	1.57±0.07(3.69)	1.57±0.09(11.30)	1.55±0.06**(18.42)	1.51±0.06***(22.96)	1.45±0.03***(31.92)
13 (10)	0.88±0.05	1.52±0.11(6.75)	1.61±0.04(9.04)	1.70±0.04(10.53)	1.52±0.09***(22.45)	1.49±0.07***(30.05)
13 (20)	0.90±0.05	1.28±0.06**(21.47)	1.42±0.05***(19.77)	1.47±0.04***(22.63)	1.32±0.05***(32.65)	1.25±0.02***(41.31)
Diclofenac (20)	0.89±0.04	1.22±0.05***(25.15)	1.25±0.05***(29.38)	1.30±0.03***(31.58)	1.16±0.02***(40.82)	1.13±0.03***(46.94)

Values are given as mean ± S.E.M. for groups of six animals each, Dunnet's test; values are statistically significantat ^{a}P<0.001, ^{b}P<0.01, ^{c}P<0.05 between control and treated groups

4. DISCUSSION

Embelin isolated from *Embelia ribes* is known for its potent biological properties [4-11]. Our earlier studies reported potent antioxidant, analgesic and anti-inflammatory activities of embelin derivatives [18]. Continuation of the earlier studies, in the present study, seven new and six known embelin derivatives were prepared and characterized. All these compounds were screened for their *in vitro* antioxidant activity using standard ABTS and DPPH methods.

When the phenylalanine (9), phenylhydrazine (10), 2,4-dinitro phenyl hydrazine (11), and DL-tryptophan (12) were substituted at C-5 of embelin, the activity was found to be more than embelin in both the methods. The addition of hydrazines and amino acids was found to be beneficial for the activity. Compound 13 was also found to be more active in both the methods indicating phenazine formation is beneficial for the activity. Among all the derivatives, hydrazines, amino acids substitutions (9-12) and phenazine nucleus (13) showed more active than embelin in both the antioxidant methods. Based on these results, compounds 9-13 along with 1 were chosen for comparing their *in vivo* analgesic and anti-inflammatory activities with embelin.

In the present study analgesic activity of embelin and its derivatives (1 and 9-13) was evaluated by hot plate, tail immersion and acetic acid induced writhing methods. These tests allows to analyze peripheral and centrally mediated antinociceptive responses. Hot plate test and tail withdrawal response has selectivity for opioid derived centrally mediated analgesics [25]. Animals treated with embelin or its derivatives showed significantly longer latency than the control group in both the methods indicating that these compounds cause analgesia by their actions at CNS. Acetic acid causes an increase in peritoneal fluids of PGE_2 and $PGF_{2\alpha}$, serotonin and histamine involved in part, which is a model commonly used for screening peripheral analgesics [26]. All the six compounds abolished the acetic acid induced writhing at both the doses indicating their potent activity by peripheral antinociceptive action. This result indicates that the analgesic effect of compounds 1 and 9-13 might be mediated by its peripheral effects by inhibiting the synthesis or action of prostaglandins [27].

Carrageenan induced inflammation is a non-specific inflammation resulting from a complex of diverse mediators [24]. This model is conventional, sensitive, accepted for screening of newer anti-inflammatory agents and reliably predicts the anti-inflammatory efficacy based on inhibition of prostaglandin amplification. In the present study, compound 9 and 13 exhibited potent effect indicating it to be a good candidate for anti-inflammatory activity. The potent activity may be due to the presence of aminoacid nucleus and phenazine formation. The observed potent analgesic and anti-inflammatory properties of embelin derivatives in the present study may be due to their potent antioxidant nature.

5. CONCLUSION

In conclusion, we have synthesized a series of embelin derivatives (2-13) and tested for *in vitro* antioxidant activity. Compounds 9-13 displayed promising radical scavenging activity among the synthesized compounds. The present data suggest that the phenyl alanine substituted compound 9 and phenazine formed compound 13 possessed potent analgesic and anti-inflammatory activities than the parent compound embelin. Further research would be of interest to explain the exact mechanism of these compounds and chemical modifications, biological screening and toxicity studies can also be explored.

CONSENT

Not applicable.

ETHICAL APPROVAL

Animal studies were performed according to the prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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