Synthesis and Evaluation of Analgesic and Anti-inflammatory Activities of Most Active Free Radical Scavenging Derivatives of Embelin —A Structure–Activity Relationship

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Antioxidant and related properties of the plant *Embelia ribes* and embelin are well known. In the present study embelin was condensed with various aromatic substituted primary amines to yield ten new and one reported derivatives along with monomethyl embelin. All these compounds along with embelin were evaluated for *in vitro* antioxidant activity using 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2,2′-diphenyl-1-picryl hydrazyl (DPPH) methods. Two *para*-substituted embelin derivatives 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 both of its derivatives almost completely abolished the acetic acid induced writhing. *p*-Sulfonylamine phenylamino derivative showed better anti-inflammatory activity than embelin.

Key words embelin; structure–activity relationship; antioxidant; analgesic; anti-inflammatory

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 central nervous system (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)]

Since, embelin exhibited potent antioxidant, analgesic and anti-inflammatory activities, in the present study a comparative evaluation of embelin and its synthetic derivatives for *in vitro* antioxidant activity using standard 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2,2′-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging methods was carried out. The potent compounds were subjected to *in vivo* analgesic and anti-inflammatory screening in experimental animals.

Results

Chemistry Embelin on methylation gave monomethyl embelin (**2**) and condensation with aniline gave di-imine (**3**). The structure of embelin derivatives is shown in Fig. 1. Condensation of embelin with various substituted aromatic primary amines, 3-nitro aniline (**4**), 4-nitro aniline (**5**), 4-chloro aniline (**6**), 2-chloro aniline (**7**), *p*-anisidine (**8**), 3-amino phenol (**9**), 4-amino phenol (**10**), *p*-toluidine (**11**), 2-amino

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benzamide (**12**) and sulfanilamide (**13**) gave ten new compounds. All these compounds were characterized by spectral studies. 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[18] 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

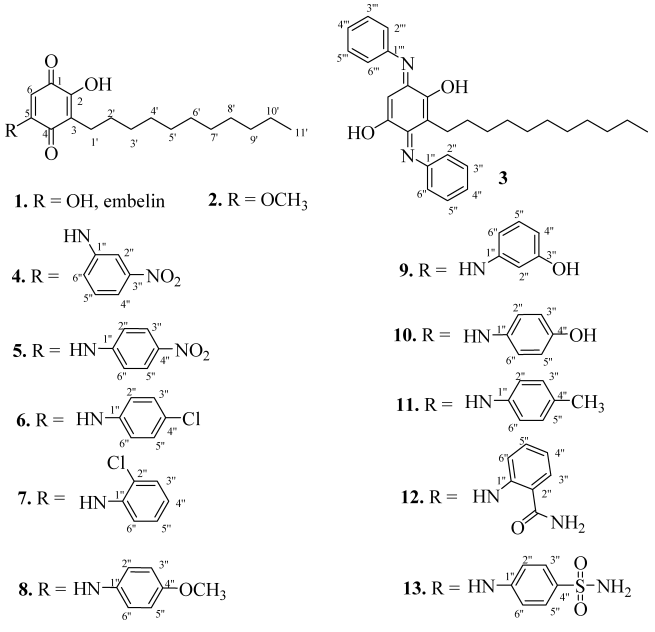

Fig. 1. The Structure of Embelin and Its Synthetic Derivatives

^{13}C-NMR of 2,5-dihydroxy-3-alkyl-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.[19] The fluxional effect can be precluded if at least one of the hydroxyl group is removed through structural modification. Such modifications led to observation of all the ring carbons except in compounds **4**, **5**, **9** and **12** as shown in Table 1. The IR, ^{1}H-NMR and mass spectral data of these compounds confirmed their structures.

Methylation of **1** with dimethyl sulphate produced **2**, which exhibited a pseudomolecular ion at *m/z* 307 for the $[M-H]^{-1}$, corresponding to the addition of one methyl group at 5th position. The IR spectra of **2** showed absorptions at 3352 cm^{-1} for –OH group, 2918, 2850 cm^{-1} for aliphatic –CH stretching, 1635 cm^{-1} for α,β-unsaturated C=O, 1599 cm^{-1} is due to the presence of C=C, and at 1079 cm^{-1} for C–O groups. ^{1}H-NMR spectrum of **2** showed a signal at δ 3.85 (s, 3H) for the methoxy group. The signal at δ 7.80 (1H, br s) for one phenolic hydroxyl group at C-2 position and at δ 5.80 for H-6 were assigned. The signal at δ 0.87 (t, 3H, H-11′), the broad singlet at δ 1.56 (18H, H-2′—H-10′) and a signal at δ 2.46 (t, 2H, H-1′) showed the presence of the undecyl chain at C-3 position of embelin. The methoxy carbon signal at δ 56.71 and the two carbonyl carbon signals at δ 182.82 (C-1) and 181.67 (C-4) were also observed in ^{13}C-NMR spectra (Table 1).

Compound **3**, exhibited a molecular ion at *m/z* 443 for the $[M-H]^-$ corresponding to the formation of a di-imine substituted compound. Accordingly, absence of α,β-unsaturated carbonyl absorption at 1630 cm^{-1} and appearance of absorption at 1581 cm^{-1} for C=N in the IR spectra manifested the

Table 1. ^{13}C-NMR Values for Embelin and Its Derivatives

Carbon	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*a)*	182.82	170.10	177.73	*a)*	182.73	182.66	182.94	*a)*	183.29	182.95	182.79	183.16
2	*a)*	161.14	153.76	*a)*	153.64	154.17	153.89	154.61	*a)*	156.66	154.45	153.49	155.98
3	116.99	119.29	113.05	117.10	117.39	116.54	116.81	116.05	114.94	116.19	116.16	117.10	117.22
4	*a)*	181.67	170.10	180.88	181.18	180.45	180.79	179.91	180.59	180.02	180.13	*a)*	181.40
5	*a)*	151.51	153.76	145.30	143.22	145.73	145.14	146.88	154.29	147.48	146.31	145.27	145.33
6	102.15	102.15	84.36	96.15	97.76	94.90	95.59	93.62	94.95	93.94	94.13	96.05	97.19
Chain 1′	22.51	22.50	22.69	22.00	22.50	22.68	22.67	22.68	22.68	22.54	22.67	22.51	22.43
to	to	to	to	to	to	to	to	to	to	to	to	to	to
10′	31.90	31.89	31.64	31.90	31.89	31.91	31.91	31.91	31.91	31.74	31.91	31.90	31.74
11′	14.09	14.08	14.10	14.09	14.08	14.10	14.09	14.09	14.10	14.41	14.09	14.09	14.40
1″	—	—	137.25	145.30	132.50	131.66	130.51	129.55	138.20	129.27	136.37	132.59	141.63
2″	—	—	123.29	127.96	125.63	129.90	127.85	124.73	109.66	126.00	122.79	128.41	117.85
3″	—	—	129.73	148.78	121.18	123.99	126.76	114.93	154.29	116.37	130.26	124.34	123.21
4″	—	—	127.40	127.96	134.58	135.56	134.17	158.07	113.39	155.89	134.28	138.33	140.32
5″	—	—	129.73	130.68	121.18	123.99	123.20	114.93	130.74	116.37	130.26	132.59	127.38
6″	—	—	123.29	120.45	125.63	129.90	127.73	124.73	114.94	126.00	122.79	122.01	117.22
1‴	—	—	137.25	—	—	—	—	—	—	—	—	—	—
2‴	—	—	123.29	—	—	—	—	—	—	—	—	—	—
3‴	—	—	129.73	—	—	—	—	—	—	—	—	—	—
4‴	—	—	127.40	—	—	—	—	—	—	—	—	—	—
5‴	—	—	129.73	—	—	—	—	—	—	—	—	—	—
6‴	—	—	123.29	—	—	—	—	—	—	—	—	—	—
5-OCH_3	—	56.71	—	—	—	—	—	—	—	—	—	—	—
4″-OCH_3	—	—	—	—	—	—	—	55.55	—	—	—	—	—
4″-CH_3	—	—	—	—	—	—	—	—	—	—	20.99	—	—
2″-$CONH_2$	—	—	—	—	—	—	—	—	—	—	—	170.05	—

a) Carbon peaks not appeared due to fluxional effect.

change from carbonyl to di-imine group. In the ^{1}H-NMR spectrum of **3**, the signals at δ 7.45 (br s, 2H) was assigned to two phenolic hydroxyl groups at C-2 and C-5 positions, δ 6.07 to H-6 and δ 7.19—7.40 (m, 10H) was assigned for ten aromatic protons. The remaining signals in the upfield region due to undecyl chain were similar to the parent compound **1**. The ^{13}C-NMR spectrum of **3** showed signals at δ 170.10 assigned for C=N at positions 1 and 4. The signals between δ 123.29—137.25 were assigned for aromatic carbons and the remaining carbon signals were similar to the parent compound **1**.

All the other compounds (**4**—**13**) showed pseudomolecular ions for the $[M-H]^-$ ion in the mass spectra at *m/z* 413 (**4**, **5**), 402 (**6**, **7**), 398 (**8**), 384 (**9**, **10**), 382 (**11**), 411 (**12**) and 447 (**13**), respectively. The IR spectra of compounds **4**—**13** 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 at 1550—1620 cm^{-1} due to the presence of aromatic C=C. Complementing these data, the presence of two carbonyl bands at δ 180 to 184 indicated that the carbonyl group remained and undisturbed in these molecules.

In ^{1}H-NMR spectrum of **4**—**13**, –NH proton was observed at δ 7.25—9.61 and H-6 was observed at δ 5.40 to 6.10. The signals between δ 7.00—8.20 were assigned for aromatic protons and the rest of the signals between δ 0.86—2.50 were due to the presence of aliphatic chain. In ^{13}C-NMR spectrum of **4**—**13**, aromatic carbons appeared between δ 113.39—158.07, C-6 appeared between δ 93.62—97.76 and C-5 appeared at δ 138.00 to 147.48. The C-5 signal appeared at δ 151.80 to 154.61 for compounds **1**—**3**. The reason for the difference may be attributed to the fact that in compounds **1**—**3** oxygen function is bonded to C-5, whereas in compounds **4**—**13** nitrogen is bonded to C-5. These data 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.

Further in ^{1}H-NMR spectrum of compound **8**, the peak present at δ 3.87 (s, 3H) is assigned for the presence of –OCH_3 group and it exhibited a signal at δ 55.55 in ^{13}C-NMR. Similarly, due to the presence of –CH_3 group in compound **11**, it showed the signal at δ 2.36 in the ^{1}H-NMR spectrum and at δ 20.99 in ^{13}C-NMR. In compound **12**, signal at δ 10.79 (s, 2H, –$CONH_2$) in ^{1}H-NMR and at δ 170.05 for C=O in ^{13}C-NMR showed the presence of amide group. In compound **13**, the signal exhibited at δ 6.59 (s, 2H, –NH_2) was due to the –NH_2 group present in sulfanilamide. The region-chemistry of the compounds **4**—**13** is tentatively assigned.

***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,[20] respectively. The *p*-hydroxy phenylamino derivative of embelin, **10** exhibited potent antioxidant activity better than embelin with IC_{50} values 0.18±0.02 μg/ml in ABTS and 25.96±1.73 μg/ml in DPPH methods, respectively. Compound **13** also exhibited similar results in ABTS method with IC_{50} value of 0.20±0.07 μg/ml. 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**, **2**, **5**, **9**, **10**, **12** and **13** (Table 2).

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 **10** were found to possess potent and **5**, **8**, **9** and **13** moderate antioxidant activities. Based on these results, compounds **10** and **13** along with **1** were chosen for comparing their *in vivo* analgesic and anti-inflammatory activities with embelin.

Analgesic Activity In the hot plate method of analgesic activity,[21] embelin and both of its derivatives exhibited potent activity. The response time observed for the all the three compounds was significantly increased when compared to normal control (Fig. 2). The activity observed was found to be higher than the standard pentazocine after 15 min for compound **10** at 10 and 20 mg/kg, and for compound **13** at 20 mg/kg. Similar results were observed for both the compounds **10** and **13** at 20 mg/kg after 30 min. 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 66.62 to 74.15%. Compounds **10** and **13** were found to be better active than embelin.

In the tail immersion[22] and acetic acid induced writhing[21] methods, all the three 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 all the compounds except embelin at 10 mg/kg in tail immersion method (Fig. 3). The activity was found to be higher than the standard pentazocine for all the

Table 2. *In Vitro* Antioxidant Activity of Embelin Derivatives by Using ABTS and DPPH Methods

Compound	IC_{50} values±S.E.M. (μg/ml) by methods[a]		Compound	IC_{50} values±S.E.M. (μg/ml) by methods[a]	
	ABTS	DPPH		ABTS	DPPH
1	0.23±0.04	27.92±0.33	**9**	0.42±0.18	50.70±4.28
2	0.50±0.07	97.20±3.59	**10**	0.18±0.02	25.96±1.73
3	2.82±0.32	>250	**11**	1.44±0.28	>250
4	1.86±0.25	>250	**12**	0.43±0.12	111.00±3.21
5	0.34±0.11	52.40±2.78	**13**	0.20±0.07	30.40±2.79
6	3.54±0.53	>250	Ascorbic acid	11.25±0.49	4.92±0.28
7	2.99±0.22	>250	Rutin	0.52±0.04	8.91±0.10
8	1.08±0.13	59.30±1.98			

a) Average of three determinations.

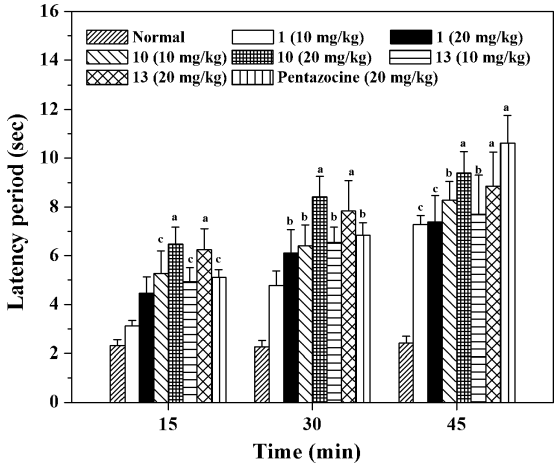

Fig. 2. Effect of Compounds **1**, **10** and **13** on Hot Plate-Induced Pain in Mice

Values are given as mean±S.E.M. for groups of six animals each, Dunnet's test; values are statistically significant at [a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ between normal and treated groups.

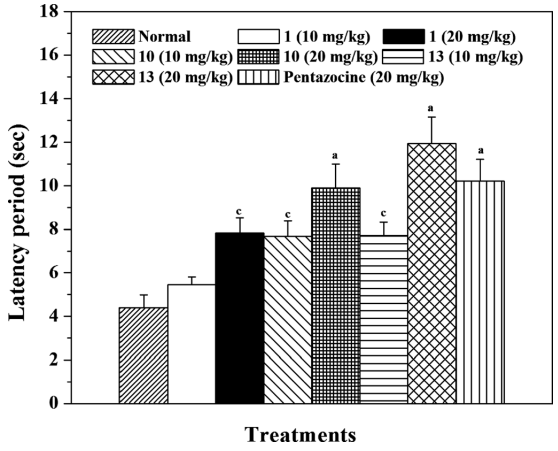

Fig. 3. Effect of Compounds **1**, **10** and **13** on Tail Immersion-Induced Pain in Mice

Values are given as mean±S.E.M. for groups of six animals each, Dunnet's test; values are statistically significant at [a] $p<0.001$ and [c] $p<0.05$ between normal and treated groups.

compounds in acetic acid induced writhing, and in tail immersion method for compound **13** at 20 mg/kg. Both compounds **10** 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 three compounds in the acetic acid induced writhing method (Fig. 4). At higher dose, all the compounds almost completely abolished the writhing indicating their potent analgesic activity.

Anti-inflammatory Activity Against carrageenan induced paw edema in rats,[23] embelin given intraperitoneally (i.p.) at 20 mg/kg significantly reduced the paw edema after 120, 180 and 360 min when compared to control (Table 3). Compounds **10** and **13** exhibited significant activity at 10 mg/kg after the 360 min and compound **10** at 20 mg/kg after 180 and 360 min. However, compound **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.

Discussion

Embelin isolated from *Embelia ribes* is known for its po-

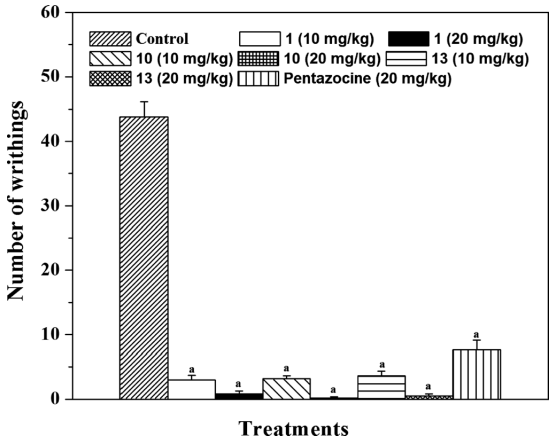

Fig. 4. Effect of Compounds **1**, **10** and **13** on Acetic Acid-Induced Writhing in Mice

Values are given as mean±S.E.M. for groups of six animals each, Dunnet's test; values are statistically significant at [a] $p<0.001$ between control and treated groups.

Table 3. Effect of Compounds **1**, **10** and **13** on Carrageenan Induced Paw Edema

Treatments (dose, mg/kg, i.p.)	Paw volume, ml after min (% protection)					
	0	30	60	120	180	360
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)
10 (10)	0.96±0.04	1.60±0.12 (1.84)	1.70±0.11 (3.95)	1.84±0.11 (3.15)	1.83±0.11 (6.63)	1.74±0.11*** (18.31)
10 (20)	0.89±0.06	1.49±0.03 (8.59)	1.69±0.04 (4.50)	1.74±0.03 (8.42)	1.60±0.07*** (18.31)	1.52±0.03* (28.64)
13 (10)	0.85±0.07	1.55±0.08 (4.90)	1.71±0.08 (3.39)	1.73±0.06 (8.95)	1.75±0.13 (10.71)	1.63±0.15** (23.47)
13 (20)	0.92±0.07	1.32±0.07*** (19.02)	1.44±0.05** (18.64)	1.62±0.04*** (14.74)	1.49±0.05* (23.98)	1.36±0.07* (36.15)
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.

tent biological properties.[4—11] Chemical modification of embelin for obtaining compounds with better activity has been tried. In the present study, ten new and two known embelin derivatives were prepared and characterized. All these compounds were screened for their *in vitro* antioxidant activity using standard ABTS and DPPH methods.

Methylation of embelin, reduced its activity in both the methods indicating that phenolic –OH group of embelin at C-5 is essential for the activity. Similarly, when the di-ketone groups at C-1 and C-4 in embelin were substituted with aniline to form a di-imine derivative, the activity was found to be decreased. Hence, the quinone moiety of embelin is again essential for the activity. When the *p*-hydroxy aniline is substituted at C-5 of embelin (**10**), the activity was found to be more than embelin in both the methods. The addition of polar amino and phenolic groups was found to be beneficial for the activity. Compound **13** was found to be more active in ABTS method than embelin indicating that the substitution of *p*-sulfonylamine phenylamino group helped to improve the activity. Among all the *para* substituted phenyl derivatives, compound **10** in both the methods and **13** in ABTS method were found to be more active than embelin (**1**). Based on these results, it can be concluded that the *para* position of aromatic amine may be a better place for introducing subtituents than *meta* and *ortho* positions. These two compounds (**10**, **13**) were selected for screening of analgesic and anti-inflammatory activities.

In the present study analgesic activity of embelin and its derivatives (**1**, **10**, **13**) was evaluated by hot plate, tail immersion and acetic acid induced writhing methods. These tests allow to analyze peripheral and centrally mediated antinociceptive responses. Hot plate test and tail withdrawal response has selectivity for opioid derived centrally mediated analgesics.[24] 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 prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$, serotonin and histamine involved in part, which is a model commonly used for screening peripheral analgesics.[25] All the three 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**, **10** and **13** might be mediated by its peripheral effects by inhibiting the synthesis or action of prostaglandins. Furthermore it is assumed that the compound **10** has some similarity to the structure of analgesics paracetamol (acetaminophen). This may or may not be related to the activity. The activity was found to be better than standard pentazocine.

Carrageenan induced inflammation is a non-specific inflammation resulting from a complex of diverse mediators.[23] 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 **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 *p*-sulfonamide nucleus in the molecule. The observed potent analgesic and anti-inflammatory properties of embelin derivatives in the present study may be due to their potent antioxidant nature.

In conclusion, we have synthesized a series of embelin derivatives (**2**—**13**) and tested for *in vitro* antioxidant activity. Compounds **10** and **13** displayed promising radical scavenging activity among the synthesized compounds. The present data suggest that the *p*-substituted compounds **10** and **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.

Experimental

General IR spectrum was recorded using Fourier transform (FT)-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., CA, U.S.A., 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-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and carrageenan were obtained from Sigma Aldrich Co., St. Louis, U.S.A. Pentazocine was obtained from Ranbaxy, New Delhi, India. Diclofenac sodium was obtained from Wochardt Ltd., Mumbai, India. Rutin was obtained from Acros Organics, NJ, U.S.A. Ascorbic acid was obtained from S.D. Fine Chem, Ltd., Biosar, India. All other chemicals used were of analytical grade.

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, Tamilnadu, India, where a voucher specimen has been deposited for further reference.

Extraction and Isolation of Embelin (1) Coarsely powdered berries of *Embelia ribes* (2 kg) were exhaustively extracted with *n*-hexane by cold extraction method (3×2 l). After 72 h, the extracts were concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40—50 °C). The residue so obtained was subjected to column chromatography over silica gel (100—200 mesh) and elution with benzene yielded an orange coloured powder,[26] which on crystallization with ether afforded orange plates of embelin **1** (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,[18] mp 141—143 °C; IR ν_{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′, J=6.9 Hz), 1.47 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.88 (t, 3H, H-11′, J=6.9 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative electrospray ionization (ESI)-MS: *m*/*z* Calcd for 294.18, Found: 293 $[M-H]^{-1}$.

Methylation of Embelin (2) Embelin (0.294 g, 1 mmol) was refluxed with dimethylsulphate (630 mg, 5 mmol) and anhydrous potassium carbonate (2 g) in dry acetone (100 ml) for 30 h. The product (2-hydroxy-5-methoxy-3-undecyl-1,4-benzoquinone) was crystallized from alcohol as deep yellow rectangular tablets,[27] mp 88—90 °C; yield 0.273 g, 88.63%; IR ν_{max} (KBr) cm^{-1}: 3352 (O–H), 2918, 2850 (C–H), 1635 (α,β-unsaturated C=O), 1599 (C=C), 1444, 1323, 1207, 1112, 839, 686; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.80 (s, 1H, –OH), 5.80 (s, 1H, H-6), 3.85 (s, 3H, $-OCH_3$), 2.46 (t, 2H, H-1′, J=7.1 Hz), 1.56 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.87 (t, 3H, H-11′, J=7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative ESI-MS: *m*/*z* Calcd for 308.20, Found: 307 $[M-H]^{-1}$.

Condensation of Embelin with Primary Amines. General Procedure Embelin (0.294 g, 1 mmol) and the primary amine (1 mmol) were boiled under reflux on a water bath at 100 °C for 2—3 h.[14,15] The cooled reaction mixture 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 (**3**—**13**).

3,6-Bis(phenylimino)-2-undecylcyclohexa-1,4-diene-1,4-diol (3) Obtained as green prisms, mp 192—194 °C; yield 0.352 g, 79.28%; IR ν_{max} (KBr) cm^{-1}: 3109 (O–H), 2920, 2850 (C–H), 1599 (C=C), 1518 (C=N),

1494, 1435, 1390, 1228, 827, 686; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.45 (s, 2H, –OH), 7.19—7.40 (m, 10H, aromatic), 6.07 (s, 1H, H-6), 2.49 (t, 2H, H-1′, J=7.3 Hz), 1.52 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.87 (t, 3H, H-11′, J=7.3 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative ESI-MS: *m/z* Calcd for 444.28, Found: 443 $[M-H]^{-1}$.

5-(3-Nitrophenylamino)-2-hydroxy-3-undecyl-1,4-benzoquinone (4) Obtained as reddish brown rectangular prisms, mp 199—201 °C; yield 0.391 g, 94.44%; IR ν_{max} (KBr) cm^{-1}: 3308 (O–H), 3248 (N–H), 2918, 2850 (C–H), 1635 (α,β-unsaturated C=O), 1616 (C=C), 1504, 1348 (NO_2), 1222, 1111, 829, 732; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.12 (t, 1H, H-6″, J=6.9 Hz), 8.08 (dt, 1H, H-4″, J=7, 6.9 Hz), 8.00 (s, 1H, –NH), 7.80 (s, 1H, –OH), 7.60 (t, 1H, H-5″, J=6.9 Hz), 7.58 (t, 1H, H-2″), 6.08 (s, 1H, H-6), 2.47 (t, 2H, H-1′, J=7.1 Hz), 1.53 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.88 (t, 3H, H-11′, J=7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative ESI-MS: *m/z* Calcd for 414.53, Found: 413 $[M-H]^{-1}$.

5-(4-Nitrophenylamino)-2-hydroxy-3-undecyl-1,4-benzoquinone (5) Obtained as brown prisms, mp 168—170 °C; yield 0.368 g, 88.89%; IR ν_{max} (KBr) cm^{-1}: 3309 (O–H), 3261 (N–H), 2920, 2848 (C–H), 1643 (α,β-unsaturated C=O), 1616 (C=C), 1504, 1352 (NO_2), 1220, 1116, 769, 696; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.30 (d, 2H, H-3″, 5″, J=7.2 Hz), 8.15 (s, 1H, –NH), 7.65 (s, 1H, –OH), 7.40 (d, 2H, H-2″, 6″, J=7.1 Hz), 6.00 (s, 1H, H-6), 2.40 (t, 2H, H-1′, J=7.0 Hz), 1.56 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.86 (t, 3H, H-11′, J=7.0 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative ESI-MS: *m/z* Calcd for 414.22, Found: 413 $[M-H]^{-1}$.

5-(4-Chlorophenylamino)-2-hydroxy-3-undecyl-1,4-benzoquinone (6) Obtained as violet prisms, mp 176—178 °C; yield 0.330 g, 82.13%; IR ν_{max} (KBr) cm^{-1}: 3319 (O–H), 3240 (N–H), 2920, 2848 (C–H), 1637 (α,β-unsaturated C=O), 1572 (C=C), 1381, 1217, 1172, 817, 707; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.90 (s, 1H, –NH), 7.85 (s, 1H, –OH), 7.40 (d, 2H, H-2″, 6″, J=7.1 Hz), 7.20 (d, 2H, H-3″, 5″, J=7.2 Hz), 5.94 (s, 1H, H-6), 2.42 (t, 2H, H-1′, J=7.2 Hz), 1.53 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.89 (t, 3H, H-11′, J=7.2 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative ESI-MS: *m/z* Calcd for 403.47, Found: 402 $[M-H]^{-1}$.

5-(2-Chlorophenylamino)-2-hydroxy-3-undecyl-1,4-benzoquinone (7) Obtained as dark violet rectangular prisms, mp 122—124 °C; yield 0.248 g, 61.54%; IR ν_{max} (KBr) cm^{-1}: 3308 (O–H), 3267 (N–H), 2922, 2854 (C–H), 1637 (α,β-unsaturated C=O), 1572 (C=C), 1379, 1213, 1060, 831, 752; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.20 (s, 1H, –NH), 7.80 (s, 1H, –OH), 7.48 (dd, 1H, H-6″, J=7.0, 2.0 Hz), 7.40 (dd, 1H, H-3″, J=, 2.0 Hz), 7.32 (td, 1H, H-5″, J=7.0, 2.0 Hz), 7.19 (td, 1H, H-4″, J=7.0, 2.0 Hz), 6.00 (s, 1H, H-6), 2.50 (t, 2H, H-1′, J=7.1 Hz), 1.50 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.90 (t, 3H, H-11′, J=7.2 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative ESI-MS: *m/z* Calcd for 403.19, Found: 402 $[M-H]^{-1}$.

5-(4-Methoxyphenylamino)-2-hydroxy-3-undecyl-1,4-benzoquinone (8) Obtained as brown prisms, mp 173—175 °C; yield 0.360 g, 90.22%; IR ν_{max} (KBr) cm^{-1}: 3313 (O–H), 3230 (N–H), 2918, 2850 (C–H), 1637 (α,β-unsaturated C=O), 1612, 1572 (C=C), 1519, 1498, 1219, 1031, 821, 711; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.05 (s, 1H, –NH), 7.95 (s, 1H, –OH), 7.22 (d, 2H, H-2″, 6″, J=7.1 Hz), 6.99 (d, 2H, H-3″, 5″, J=7.1 Hz), 5.88 (s, 1H, H-6), 3.87 (s, 3H, $-OCH_3$), 2.51 (t, 2H, H-1′, J=7.2 Hz), 1.59 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.93 (t, 3H, H-11′, J=7.2 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative ESI-MS: *m/z* Calcd for 399.24, Found: 398 $[M-H]^{-1}$.

5-(3-Hydroxyphenylamino)-2-hydroxy-3-undecyl-1,4-benzoquinone (9) Obtained as black amorphous solid, mp 170—173 °C; yield 0.321 g, 83.38%; IR ν_{max} (KBr) cm^{-1}: 3317 (O–H), 3240 (N–H), 2918, 2848 (C–H), 1637 (α,β-unsaturated C=O), 1604, 1570 (C=C), 1519, 1508, 1381, 1217, 1151, 829, 707. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.05 (s, 1H, 3″-OH), 9.55 (s, 1H, –NH), 7.90 (s, 1H, –OH), 6.81 (dd, 1H, H-6″, J=6.9, 2.1 Hz), 6.74 (t, 2H, H-2″, 5″, J=7.0 Hz), 6.70 (dd, 1H, H-4″, J=7.1, 1.9 Hz), 6.04 (s, 1H, H-6), 2.44 (t, 2H, H-1′, J=7.2 Hz), 1.50 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.89 (t, 3H, H-11′, J=7.2 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative ESI-MS: *m/z* Calcd for 385.24, Found: 384 $[M-H]^{-1}$.

5-(4-Hydroxyphenylamino)-2-hydroxy-3-undecyl-1,4-benzoquinone (10) Obtained as brown amorphous solid, mp 180—182 °C; yield 0.277 g, 71.95%; IR ν_{max} (KBr) cm^{-1}: 3313 (O–H), 3232 (N–H), 2920, 2848 (C–H), 1635 (α,β-unsaturated C=O), 1614, 1570 (C=C), 1519, 1504, 1383, 1219, 1112, 823, 709; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.58 (s, 1H, 4″-OH), 9.61 (s, 1H, –NH), 7.83 (s, 1H, –OH), 7.12 (d, 2H, H-2″, 6″, J=7.1 Hz), 6.81 (d, 2H, H-3″, 5″, J=7.0 Hz), 5.49 (s, 1H, H-6), 2.42 (t, 2H, H-1′, J=7.1 Hz), 1.46 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.87 (t, 3H, H-11′, J=7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative ESI-MS: *m/z* Calcd for 385.24, Found: 384 $[M-H]^{-1}$.

5-(*p*-Tolylamino)-2-hydroxy-3-undecyl-1,4-benzoquinone (11) Obtained as deep violet prisms, mp 147—149 °C; yield 0.350 g, 91.38%; IR ν_{max} (KBr) cm^{-1}: 3302 (O–H), 3244 (N–H), 2920, 2848 (C–H), 1641 (α,β-unsaturated C=O), 1608, 1572 (C=C), 1518, 1496, 1384, 1222, 1205, 813, 719; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.45 (s, 1H, –NH), 7.91 (s, 1H, –OH), 7.20 (d, 2H, H-3″, 5″, J=6.9 Hz), 7.11 (d, 2H, H-2″, 6″, J=6.9 Hz), 5.93 (s, 1H, H-6), 2.44 (t, 2H, H-1′), 2.36 (s, 3H, 4″-CH_3, J=7.1 Hz), 1.46 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.86 (t, 3H, H-11′, J=7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative ESI-MS: *m/z* Calcd for 383.25, Found: 382 $[M-H]^{-1}$.

2-(4-Hydroxy-3,6-dioxo-5-undecylcyclohexa-1,4-dienylamino)benzamide (12) Obtained as dark brown solid, mp 127—129 °C; yield 0.327 g, 79.37%; IR ν_{max} (KBr) cm^{-1}: 3171, 3090 (–NH_2), 3308 (O–H), 3227 (N–H), 2918, 2850 (C–H), 1637 (α,β-unsaturated C=O), 1618, 1541 (C=C), 1518, 1465, 1431, 1390, 1143, 1120, 756, 594; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.79 (s, 2H, 2″-$CONH_2$), 8.30 (s, 1H, –NH), 7.82 (s, 1H, –OH), 7.60 (d, 2H, H-5″, 6″, J=6.9 Hz), 7.53 (d, 2H, H-3″, 4″, J=6.9 Hz), 6.00 (s, 1H, H-6), 2.46 (t, 2H, H-1′, J=7.1 Hz), 1.49 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.87 (t, 3H, H-11′, J=7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative ESI-MS: *m/z* Calcd for 412.24, Found: 411 $[M-H]^{-1}$.

5-(4-Sulfonylaminephenylamino)-2-hydroxy-3-undecyl-1,4-benzoquinone (13) Obtained as black amorphous solid, mp 157—159 °C; yield 0.409 g, 91.29%; IR ν_{max} (KBr) cm^{-1}: 3375, 3277 (NH_2), 3311 (O–H), 3238 (N–H), 2920, 2848 (C–H), 1637 (α,β-unsaturated C=O), 1616, 1573 (C=C), 1508, 1465, 1327, 1220, 1163 (–SO_2), 1101, 767, 707, 543; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.10 (s, 1H, –NH), 7.96 (s, 1H, –OH), 6.59 (s, 2H, –SO_2NH_2), 7.31 (d, 2H, H-3″, 5″, J=7.0 Hz), 7.21 (d, 2H, H-2″, 6″, J=7.0 Hz), 5.99 (s, 1H, H-6), 2.42 (t, 2H, H-1′, J=7.1 Hz), 1.45 (m, 2H, H-2′), 1.25—1.30 (m, 16H, H-3′ to 10′), 0.88 (t, 3H, H-11′, J=7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1; negative ESI-MS: *m/z* Calcd for 448.58, Found: 447 $[M-H]^{-1}$.

***In Vitro* Antioxidant Activity. 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.

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.[20]

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 37 °C for 20 min, the absorbance of each solution was determined at 490 nm[20] using enzyme-linked immunosorbent assay (ELISA) reader (Bio-Rad Laboratories Inc., CA, U.S.A., Model 550).

***In Vivo* Analgesic and Anti-inflammatory Activities. 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±3 °C, 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.

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, 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.

Eddy's Hot-Plate Method Mice were treated and placed on Eddy's hot plate kept at a temperature of 55±0.5 °C. A cut off period of 15 s 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.[21]

Tail Immersion Method In this method,[22] 5 cm of the end of the mice tail was immersed in warm water maintained at 55±0.5 °C. The tail withdrawal reflex was recorded before and after 60 min following the treatments. The percentage protection was calculated as per hot plate method.

Acetic Acid Induced Writhing Method In the acetic acid induced writhing[21] 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.

Anti-inflammatory Activity. 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.[23] 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.

Statistical Analysis The values were expressed as mean±S.E.M. 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.

Conflicts of Interest Statement The authors declare that there are no conflicts of interest.

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References

1) Varier P. S., "Indian Medicinal Plants, a Compendium of 500 Species," Orient Longman (Pvt), Chennai, India, 2006, pp. 368—371.
2) Bhandari U., Jain N., Pillai K. K., *Exp. Diabetes Res.*, **15**, 1—6 (2007).
3) Kapoor V. K., Chawla A. S., Kumar M., Kumar P., *Indian Drugs*, **30**, 481—488 (1983).
4) Krishnaswamy M., Purushothaman K. K., *Indian J. Exp. Biol.*, **18**, 1359—1360 (1980).
5) Radhakrishnan N., Alam M., *Indian J. Exp. Biol.*, **3**, 70—71 (1975).
6) Chitra M., Sukumar E., Suja V., Devi C. S. S., *Chemotherapy*, **40**, 109—113 (1994).
7) Joshi R., Kamat J. P., Mukherjee T., *Chem. Biol. Interact.*, **167**, 125—134 (2007).
8) Dharmendra S., Ruchi S., Pahup S., Gupta R. S., *Basic Clin. Pharmacol. Toxicol.*, **105**, 243—248 (2009).
9) Kumara Swamy H. M., Krishna V., Shankarmurthy K., Abdul Rahiman B., Mankani K. L., Mahadevan K. M., Harish B. G., Raja Naika H., *J. Ethnopharmacol.*, **109**, 529—534 (2007).
10) Chitra M., Devi C. S. S., Sukumar E., *Fitoterapia*, **74**, 401—403 (2003).
11) Mahendran S., Thippeswamy B. S., Veerapur V. P., Badami S., *Phytomedicine*, **18**, 186—188 (2011).
12) Ravelo A. G., Estévez-Braun A., Chávez-Orellana H., Pérez-Sacau E., Mesa-Siverio D., *Curr. Top. Med. Chem.*, **4**, 241—265 (2004).
13) Waris G., Ahsan H., *J. Carcinog.*, **5**, 14—21 (2006).
14) Padmanabha Rao T. V., Venkateswarlu V., *Tetrahedron*, **20**, 969—971 (1963).
15) Gupta O. P., Ali M. M., Ray Ghatak B. J., Atal C. K., *Indian J. Physiol. Pharmacol.*, **21**, 31—39 (1977).
16) Halliwell B., *Lancet*, **334**, 721—724 (1994).
17) Sen S., Chakraborty R., Sridhar C., Reddy Y. S. R., De B., *Int. J. Pharm. Sci. Rev. Res.*, **3**, 91—100 (2010).
18) Feresin G. E., Tapia A., Sortino M., Zacchino S., de Arias A. R., Inchausti A., Yaluff G., Rodriguez J., Theoduloz C., Schmeda-Hirschmann G., *J. Ethnopharmacol.*, **88**, 241—247 (2003).
19) Midiwo J. O., Manguro Arot L. O., *Nat. Prod. Lett.*, **8**, 11—14 (1996).
20) Badami S., Prakash O., Dongre S. H., Suresh B., *Indian J. Pharmacol.*, **37**, 251—252 (2005).
21) Kulkarni S. K., "Hand Book of Experimental Pharmacology," Vallabh Prakashan, New Delhi, India, 1999, pp. 125—128.
22) Dykstra L. A., Woods J. H., *J. Pharmacol. Methods*, **15**, 263—269 (1986).
23) Prabhakar K. R., Veerapur V. P., Bansal P., Vipan K. P., Reddy K. M., Barik A., Reddy B. K., Reddanna P., Priyadarsini K. I., Unnikrishnan M. K., *Bioorg. Med. Chem.*, **14**, 7113—7120 (2006).
24) Winter C. A., Risley E. A., Nuss G. W., *Proc. Soc. Exp. Biol. Med.*, **111**, 544—547 (1962).
25) Deraedt R., Jougney S., Delevalcee F., Falhout M., *Eur. J. Pharmacol.*, **51**, 17—24 (1980).
26) Chitra M., Shyamaladevi C. S., Viswanathan S., Sukumar E., *Indian J. Pharmacol.*, **35**, 241—244 (2003).
27) Aiyar S. N., Jain M. K., Krishnamurti M., Seshadri T. R., *Phytochemistry*, **3**, 335—339 (1963).