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Synthesis, Characterisation and Biological Evaluation of 1,7- bis-(4-hydroxy-3-methoxy phenyl) - 1, 3, 6 -heptatriene-3-hydroxy, 5-imino-2-thiophenol

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Abstract: 1,7 - bis -(4-hydroxy-3-methoxy phenyl) - 1, 3, 6 – heptatriene -3- hydroxy, 5-imino- 2-thiophenol has been synthesized by condensing [curcumin (C)] 1,7 - bis-(4-hydroxy-3-methoxy phenyl) - 1, 6-heptadiene-3, 5-dione with 2-aminothiophenol in presence of ethanol at 70 ^{o}C. Elemental analysis, melting point, UV-Vis, FT-IR, ^{1}H-NMR and mass spectral data have been used to characterize the synthesized compound. The compound was utilized to test the biological activities like analgesic, anti-inflammatory, anti-ulcer and wound healing effect on Wister male albino rats and compared with standard drugs like Pentazocin, Diclofenac sodium and Ranitidine. Creams were prepared with C and C-I and the wound healing effect was studied on rats by Excision wound model method. Antimicrobial effect of the compound was studied using gram positive (*Bacillus megatrium*) and gram negative (*E. coli*) bacteria on Nutrient Agar medium and its minimum inhibitory concentration (MIC) was found as 1000µg/ml. The synthesized compound has shown significant effect on analgesic and anti-inflammatory, anti-ulcer and wound healing effect. The compound has reduced the growth of microbial organisms and potentiates the antimicrobial effect of standard antibiotics, Gentamycin and Amikacin and antagonize the effect of Cefatoxin, on the gram positive (*Bacillus megatrium*) and gram negative (*E.coli*) organisms.

Key words: Curcumin, 2-aminothiophenol, Analgesic, Anti-inflammatory, Anti-ulcer effect.

Introduction

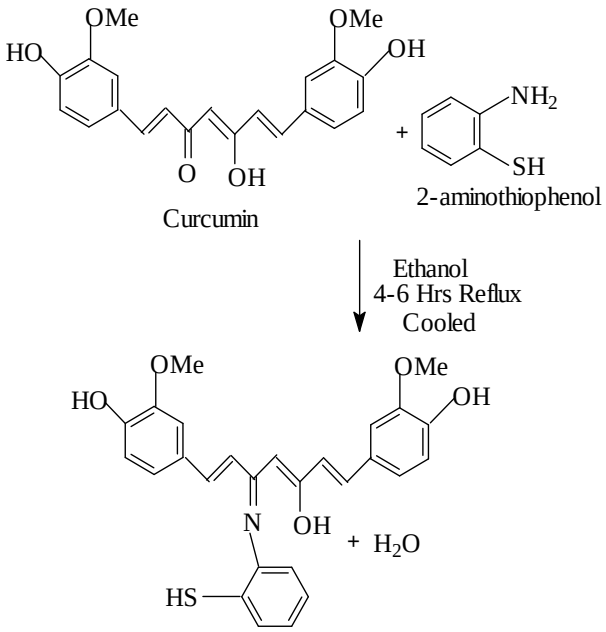
A poly phenolic compound Curcumin (1, 7 - bis-(4-hydroxy-3-methoxy phenyl) - 1, 6 -heptadiene- 3, 5-dione) is a natural yellow pigment and extracted from the root of Curcuma longa which belongs to the family of Zingiberaceae. Rhizomes are used externally in the form of paste over sprains and skin diseases[1]. Curcumin is found to possess hepatoprotective[2&3] nephroprotective[4], anti-inflammatory[5&6], ulcerprotective[7] and anti-microbial[8&9] properties. Literature survey reveals that no work has been done on the condensation between Curcumin and 2-aminothiophenol. Hence, the present work described the synthesis, characterization, analgesic and anti-inflammatory, anti-ulcer and wound healing effects of the synthesized curcuminoid on Wister male albino rats and was compared with standard drugs like Pentazocin, and Diclofenac sodium and Ranitidine. It's MIC on gram positive (*B. megatrium*) and gram negative (*E. coli*) organisms and antagonistic/synergetic effect when mixed with standard antibiotics like Gentamycin, Amikacin and Cefatoxin on the above microorganisms were studied.

Materials and Method

Merck products of the chemicals and analytical grade of solvents were used as supplied for the synthesis. The UV-Vis. spectrum of the compound was recorded on a Shimadzu UV-1604 spectrophotometer. The IR spectrum of the sample was recorded with KBr pellets using a Shimazdu 8400 Spectrophotometer. . The ^{1}H- NMR spectra of the sample was recorded on a Brucker 300 spectrometer using $CDCl_3$ as solvent. Chemical shifts (δ) are reported in ppm relative to tetramethyl silane at Madurai Kamaraj University, Madurai The animal study was approved by the animal ethical committee (509/02/C/CPCSEA/2002).

Preparation of 1, 7-bis-(4-hydroxy-3-methoxy phenyl) - 1, 3, 6 -heptatriene-3-hydroxy, 5-imino-2-thiophenol : Curcumin (0.1 mol) and 2-aminothiophenol (0.1 mol) were dissolved in 50 ml of ethanol in a round bottom flask and refluxed for 4-6 hours at 70 ^{o}C. The resulting solution was concentrated and collected in a beaker. To this mixture, 20 ml of petroleum ether (40-60 ^{o}C) was added and kept at 0 ^{o}C for about 24 hours. A dark brown solid mass obtained was collected and recrystallised in hot ethanol. Curcumin was named as C and the synthesized compound was named as C-I. Physical characterization and spectral data[10] of the synthesized compound were summarized Table – 1.

Reaction



Analgesic Activity

The analgesic activity of the synthesized compounds was carried out in albino rats by Tail flick method[11-14]. Healthy albino rats of either sex (150-200 g) were taken and divided into 4 groups (5 animals in each group). They were housed in polypropylene cages and maintained under standard conditions (12 hours light/dark cycles at (25±3^{o}C). The animals were fasted for 24 hours before the experiment. Group 1 animals were received 1 ml of 0.5 % w/v carboxy methyl cellulose solution orally (control) and group 2 animals were received Pentazocin 30 mg/kg i.p (standard). Curcumin and the synthesized drug (30 mg/kg) were suspended in the vehicle and administered orally into groups 3 and 4. The basal reaction time of each rat was noted by placing the tip of the tail of rat on the hot water at 55 ^{o}C. It was noted at different interval of time (15, 30, 45 and 60 min. after the drug treatment.) and the percentage of increase in basal reaction time was calculated and summarized in Table- 2.

Table-1. Physical Characterization and Spectral Data of the Synthesized Compound

Description		C - II
Yield (%)		70
Empirical ormula		$C_{27}H_{25}O_9SN$
M. Wt.		475
Melting point (^oC)		186
Colour		Light brown
pH		5.52
Found (calculated)	C	68.03 (68.21)
	H	5.19 (5.26)
	N	3.06 (2.95)
λ_{max} (nm)		358, 492
IR data (cm^{-1})		-OH: 3550 – 2850,-SH : 2562,-OMe: 1390, -C=N : 1582, -C=C- : 1506, aromatic ring –C=C- : 1478, - C - O- C- for OMe : 1250, enolic –C-O-: 1025,Phenolic stretch : 1330-1270., Aliphatic –C=CH stretch: 710, 750 and 810.
^{1}H NMR data (PPM)		-OMe merged (s) : 4.0, phenolic – OH (s) : 7.6, phenolic – SH (s) : 13.5 enolic – OH (s) : 11.1, active –CH (s) : 6.25, -C=CH on enolic side (d,d) 5.9 & 6.15, -C=CH on azomethine side (d,d) : 6.4, 6.7, Benzene ring in curcumin moiety (merged s,d,d) : 6.9, 7.1, 7.25, Benzene ring in 2-aminothiophenol (merged d,d) : 7.7 and 7.9.

Table-2. Analgesic Activity of Synthesized Compound by Tail Flick Method

Sample		**Basal reaction time (Mean ± SEM, sec.)**				% Index of Analgesia = $\frac{V_t - V_c}{V_t}$
	Dose/kg	**15 min.**	**30 min.**	**45 min.**	**60 min.**	
Vehicle	1ml	3.20± 0.4	2.92 ±0.14	2.54 ±1.16	2.22± 1.2	-
Standard	30mg	6.93±0.48*	6.85± .23*	7.02 ±0.42*	8.24 ±.04*	73.06
C	30mg	5.20±0.24*	5.60 ±.97*	5.88 ±1.64*	6.22± .06*	68.45
C-I	30mg	6.93 ±.90*	6.52± .02*	6.80±0.72*	7.02±0.40*	60.38

*P<0.05 is considered as significant, Vt = Value of test substance and Vc = Value of control

Anti-inflammatory Activity

The synthesized compound was utilized to test the anti-inflammatory activity by Carrageenan induced rat hind paw edema method[15-16]. Diclofenac sodium (100 mg/kg i.p.) as standard. 20 Healthy albino rats (150–200 g) were taken and divided into 4 groups (5 animals in each group). The animals were fasted for 24 hours before the experiment. The initial paw volume of each rat was found by mercury displacement method. Group 1 received 1 ml of 0.5 % w/v carboxy methyl cellulose solution orally (control) and group 2 animals were received Diclofenac sodium (100 mg/kg i.p). Curcumin and synthesized drug (100 mg/kg) were suspended in the vehicle and administered orally into groups 3 and 4 respectively. After 30 min., acute inflammation was induced in rats by injecting 0.1 ml of 1 % (w/v) solution of Carrageenan into the sub plantar region of the left paw. The paw volumes were measured at 1, 2 and 3 hours interval and the percentage protection of paw edema was calculated and summarized in Table -3.

Table-3. Anti-Inflammatory Studies of Synthesized Compound

S.		Paw volume (Mean ± SEM, ml)					
No.	Groups	Dose/kg	0 h	1 h	2 h	3 h	% protection
1	Vehicle	1 mL	0.9±0.04	1.2±0.02	1.5±0.01	1.7±0.02	-
2	Standard	100 mg/kg	1.1±0.03	1.8±0.01*	1.5±0.16*	1.2±0.01*	55.56
3	C	100 mg/kg	1.8±0.03	2±0.01*	1.2±0.2*	1.0±0.03*	48.15
4	C-I	100 mg/kg	1.6±0.03	3.2±0.01*	2.8±0.20*	1.4±0.03*	52.96

Significant *P < 0.01,

Anti-ulcer study

The anti-ulcer effect of the synthesized compound was studied by standard procedure[17-19]. Aspirin induced ulcers were treated in Wister male albino rats by suspending the synthesized compounds in 2% gum acacia solution. 20 Albino rats (150-200 g) were housed 5 in each group in a standard laboratory conditions. They were fasted for 24 hours before doing the experiment. The drugs were administered orally. Group 1 animals received 1 ml of 2% gum acacia solution and kept as control. Group 2 animals received Ranitidine 100 mg/kg i.p. (standard) Group 3 and group 4 animals received C and C-I which were suspended in gum acacia solution (100 mg/kg). After one hour, aspirin was given 100 mg/kg to each rat. After 4 hours the rats were anaesthetized and scarified. The stomachs were isolated, washed gently under clean flowing water. The gastric content was collected. The stomach was cut and opens along the greater curvature. They were fixed in 10% formalin and ulcer scores were recorded. The volume and pH of the collected gastric juice were measured. Free and total acidity were estimated by titrating it with 0.01 N standard sodium hydroxide solutions using Topfer's reagent (dimethyl-amino-azo- benzene with phenolphthalein) as indicator. When the colour turned to orange the volume of sodium hydroxide corresponds to the free acidity is measured. Further titration regain the pink colour, gave the volume of sodium hydroxide corresponding to the total acidity. The number of ulcers was counted and ulcer index (UI) was calculated (Table – 4) using the formula:

$$\text{Ulcer index} = \frac{10}{X} \quad \text{Where, } X = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}$$

Wound healing effect

Excision wound model in rats was selected for assessing the wound healing activity[20&21]. Creams were prepared by trituration method. C and C-I was mixed thoroughly with the cream base (10% w/w) separately and was used for topical application. Three groups of (5 rats in each group) of male healthy albino rats (150–200 g) of either sex were used in this experiment. They were housed individually with free access to food and water. The basal food intake and body weight was noted for each animal. The rats were anaesthetized with ether and depilated aseptically at the dorsal thoracic region before wounding. The excision wound (pointed circular area of 2.5 cm length and 0.2 cm depth) was made by using toothed forceps, and a surgical blade .The wound was left undressed and kept in an open environment. The prepared creams were applied daily. The progressive changes in the wound area were monitored by tracing the wound margin on graph paper. The wound area was measured on different interval of days (1, 5, 10, 15 and 20 days). The observed period of wound closure and the epithelium formation for all the animals were mentioned in Table-5. All the results were analyzed by using ANOVA followed by multiple range tests.

Table-4. Anti-Ulcer Effect of Synthesized Compound in Albino Rats

S.No	Treatment	pH	Free acids Meg/L	Total acidity Meg/L	Ulcer index (UI)
1	Vehicle	2.40±0.05	28.23±0.26	45.67±1.21	4.35±0.30
2	Standard	3.4*±0.12	13.2*±0.21	31.46*±0.85	0.008*±0.02
3	C	3.22*±0.19	21.4*±0.62	38.16*±0.26	3.13*±0.16
4	C-I	2.80*±0.02	22.9*±0.34	42*±0.40	3.4*±0.16

Significant *P < 0.01 No.of Animals=5

Table – 5. Wound Healing Effect of the Synthesized Drug

S. No.	No. of days	Control	C	C-I
1	1	2.5±0.12	2.7±0.82	2.6 ± 0.12
2	5	2.4±0.14	2.2 ± 0.32	2.0 ± 0.1
3	10	2.3±0.13	2.0*±0.12	1.9* ± 0.2
4	15	2.0+01	1.5*±0.42	1.3* ± 0.1
5	20	1.9+0.02	1.1±0.2	0.6* ± 0.2

Antimicrobial activity

Anti-microbial effect of the compound was tested against the bacteria, *Escherichia coli* (-) and *Bacillus megatrium* (+) by the well diffusion method[22&23] using agar nutrient medium. The stock solutions were prepared by dissolving C and C-I compounds individually in Dimethyl sulfoxide. It is serially diluted to get 100 µg/ml, 200 µg/ml, 400 µg/ml, 800 µg/ml and 1000 µg/ml and used to find the minimum inhibitory concentration. 6 mm diameter well was made on the agar medium which was previously taken in different Petri dishes and filled with test solution using micropipette. The plates were inoculated individually with the gram positive (*Bacillus megatrium*) and gram negative (*E.coli*) organisms. Thirty minutes pre-diffusion was allowed before incubation and incubated at 37^{o}C for 24 h. The zone of inhibition were observed in each plate and reported in the Table-6. All the experiments were done in triplicate.

Determination of antagonistic/synergetic effect

The standard antibiotics Cefatoxime, Gentamycin and Amikacin were mixed with solutions of the synthesized compound individually (1000µg/ml) and were tested by the above method for their antagonistic/synergetic effect using *Escherichia coli* and *Bacillus megatrium*. By observing the zone of inhibition the results were summarized in the Table-7.

Table-6. Minimum Inhibitory Concentration Effect of Synthetic Curcuminiod

Organisms	Sample	100µgm	200µgm	400µgm	800µgm	1000µg
B.megastrum	C	+	-	-	-	-
B.megastrum	C-I	+	+	+	-	-
E.coli	C	+	-	-	-	-
E.coli	C-I	+	+	+	+	-

(+) = growth; (-) = No growth

Table-7. Antagonistic / Synergetic Effect of Synthetic Curcuminoid

S.No	Antibiotics	*B. megastrum*		*E.coli*	
		C	C-I	C	C-I
1	Cefatoxime	-	-	-	-
2	Gentamycin	+	+	+	+
3	Amikacin	+	+	+	+

(+) = Synergetic Effect (-) = Antagonistic Effect

Result and Discussion

The compound was synthesized based on the chemical nature of the Curcumin by removing the unstable β-diketone moiety and preserving the phenolic OH group. Physical characterization, elemental analysis and spectral data of the synthesized compound were summarized in Table 1. From the data, the structure of the compound was confirmed as C-I and used for the determination of therapeutic effect on rats and microbes.

Analgesic effect
Tail flick method affords rapid evaluation of peripheral type of analgesic action. Analgesic action is caused by liberating endogenous substances including serotonin, histamine, Prostoglandins and bradykinin which stimulate pain nerve endings. Analgesic effect may be probably due to the inhibition of synthesis or action of prostaglandin. The synthesized Curcuminoid has less analgesic effect than C and the standard drug Pentazocin

Anti-inflammatory effect
Development of edema in the paw of the rat after injection of carrageenan is a biphasic event. During the first stage histamine, serotonin, prostaglandins, protease may be released and caused the edema. Sub plantar injection of carrageenan in rats showed a time-dependent increase in paw thickness when treated in the vehicle group. Diclofenac sodium, a standard COX-inhibitor significantly reduced the paw edema by acting against the release of histamine, serotonin and kinin. This study demonstrated that C and C-I have significant anti-inflammatory property. Compound I has more anti-inflammatory activity than C but less than the standard. It is mainly due to the presence of imino and phenyl groups.

Anti-ulcer effect
The newly synthesized Curcuminiod C-I has better effect in healing the ulcer than the Curcumin. It may be due to the scavenging effect of reactive oxygen species (ROS) or by regulating MMP activity, or both (swarnagar). These mechanisms include enhancement of the gastric mucosal defense through increase in mucus and bicarbonate production, reduction in volume of gastric acid secretion or by neutralizing the gastric acidity.

Wound healing effect
From the observed data, compound C-I showed significant reduction in wound area and the period of epithelisation. C-I treated animals showed faster epithelisation of wound than the control. The acute phase of wound healing is partially triggered by activation of platelets through the release of platelet-derived growth factor (PDGF) and eicosanoids (prostaglandins and leukotrienes), which are known to facilitate hemostasis and the inflammatory response. Platelet derived growth factor is responsible for wound reduction, enhanced cell proliferation and efficient free radical scavenging in C-I group. This study provides a productive approach to support the dermal wound healing.

Antimicrobial effect
Antimicrobial study reveals that the synthesized compound has shown that 1000µg/ml was the MIC on *Bacillus megatrium* (+), and *E. coli* (–) organisms. The result of the determination of antagonistic/synergetic effect (Table 1) demonstrated that the standard antibiotics Gentamycin, Amikacin and Cefatoxime with C and C-I produced different effects on the micro organisms ranging from synergism to antagonism. They were found to possess synergetic effect when treated with the Gentamycin and Amikacin .But when treated with Cefatoxime, C and C-I have shown antagonistic effect on both micro organisms. Although synergy was demonstrated, it is felt that further research should be done with other strains of bacteria to investigate the efficacy of the C-I as topical agents for the treatment of skin diseases of bacterial origin. Antibacterial effect may be either by inhibiting enzyme D-Alanine ligase, alanine racemase, or L-alanine ‘adding’ enzymes or by replacing both D- and L- alanine residues in peptidoglycan subunits, thereby impairing trans peptidization within the cell wall.

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