

## ANTI ULCER EFFECT OF SOME SYNTHETIC CURCUMIN DERIVATIVES

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### Summary

Curcumin has been condensed with Hydrazine/phenyl hydrazine/2, 4 -Dinitro phenyl hydrazine and alcohol to get the newer Curcuminoids. The structural features of the synthesized compounds have been determined from their elemental analysis, melting point, IR, UV-Vis, <sup>1</sup>H-NMR and mass spectral data. The compounds were utilized to test the anti ulcer effect on Wistar male albino rats, were compared with standard drug Ranitidine. The synthesized compounds were found to reduce the ulcer induced by the drug Aspirin.

**Key words:** Curcumin, Hydrazine, phenyl hydrazine,  
2,4-Dinitrophenyl hydrazine, Ranitidine and Aspirin

### Introduction

Ulcers may be produced due to natural causes (gastric cancer), infections (H. pylori), lifestyle (drugs non steroidal anti inflammatory agents, alcohol, stress and cigarette smoking) <sup>1&2</sup>. Current treatment of ulcers has been largely suppression of pain, with little or no strategy aimed to cure. Recently there has been an increasing interest in the chemistry of Curcumin because of their biological significance. A natural yellow pigment Curcumin (1,7-bis-(4-hydroxy-3-methoxy phenyl)-1,6-heptadiene- 3,5-dione) extracted from the root of *Curcuma longa* (Zingiberaceae), is considered to be a stomachic, bitter aromatic, cooling, astringent, and carminative.<sup>3-6</sup> Curcumin has protective effect against gastric ulcer development.<sup>7</sup> It is a complex agent which has intermolecular and intra molecular bonds for reactions. Hence it is taken for the condensation reaction with Hydrazine/Phenyl hydrazine/2, 4-dinito phenyl hydrazine in alcohol. The synthesized compounds were tested for their anti ulcer effect in aspirin induced wistar male albino rats and compared with standard drug Ranitidine and the parent compound Curcumin.

### Methods

All the chemicals and solvents were Merck products and spectroscopic grade were used for spectral measurements. The UV-Vis. spectra of the compounds were recorded on a Shimadzu UV-1604 spectrophotometer. The IR spectra of the samples were recorded with KBr pellets using a Shimadzu 8400 Spectrophotometer. The  $^1\text{H}$ - NMR spectra of the samples were recorded on a Bruker 300 spectrometer using  $\text{CDCl}_3$  as solvent. Chemical shifts ( $\delta$ ) are reported in ppm relative to tetramethyl silane at Madurai Kamaraj University, Madurai. Elemental analyses and mass spectral data of the samples were measured at Sophisticated Analytical Instrumentation Facilities, IIT Mumbai. The animal study was approved by the animal ethical committee (509/02/C/CPCSEA/2002).

#### Synthesis of curcumino-imino hydrazone

Curcumin (0.1 mol) and Hydrazine (0.1 mol) were dissolved in 50 ml of ethanol and refluxed at  $70^\circ\text{C}$ . The resulting solution was concentrated and collected in a beaker. To this mixture, 20 ml of petroleum ether ( $40\text{-}60^\circ\text{C}$ ) was added and kept at  $0^\circ\text{C}$  for 48 h. A light 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.

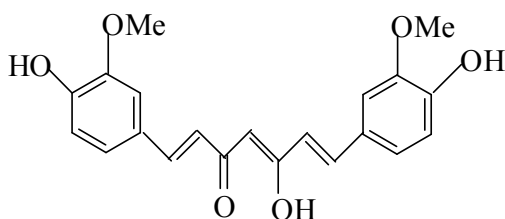
#### Synthesis of curcumino-iminophenylhydrazone

50 ml of ethanolic solution of Curcumin (0.1mol) and phenyl hydrazine (0.1mol) were taken in a round bottom flask and refluxed at  $70^\circ\text{C}$  and the resulting solution was concentrated. To this mixture, 20 ml petroleum ether ( $40\text{-}60^\circ\text{C}$ ) was added and kept at  $0^\circ\text{C}$  for 48 h. A reddish brown solid mass was collected and recrystallised in hot ethanol and named as C-II.

#### Synthesis of curcumino-imino 2-4-dinitrophenylhydrazone

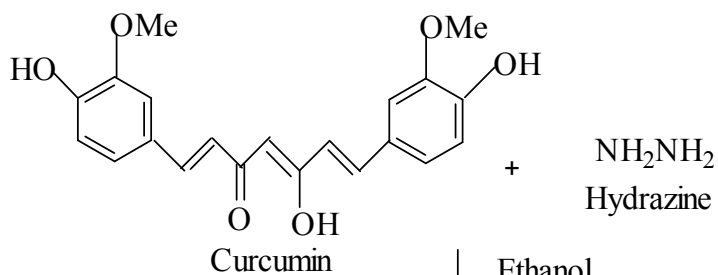
50 ml of ethanolic solution of Curcumin (0.1mol) and 2, 4 -dinitro phenyl hydrazine (0.1mol) were taken in a round bottom flask and refluxed h at  $70^\circ\text{C}$  and the resulting solution was concentrated. To this mixture, 20 ml petroleum ether ( $40\text{-}60^\circ\text{C}$ ) was added and kept at  $0^\circ\text{C}$  for 48 h. A dark brown solid mass was collected and recrystallised in hot ethanol and named as C-III. The reactions are mentioned as follows:

### Reactions

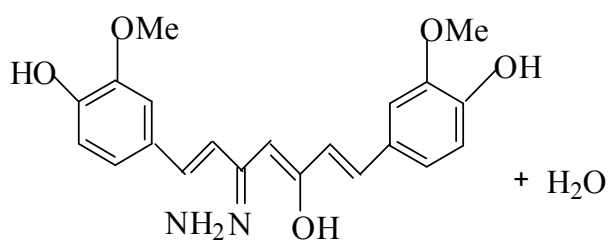


Curcumin

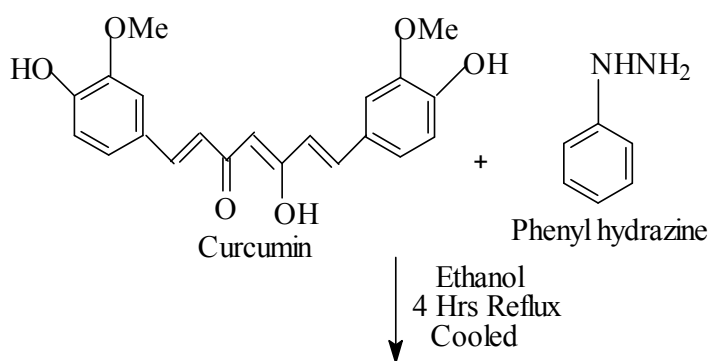
C



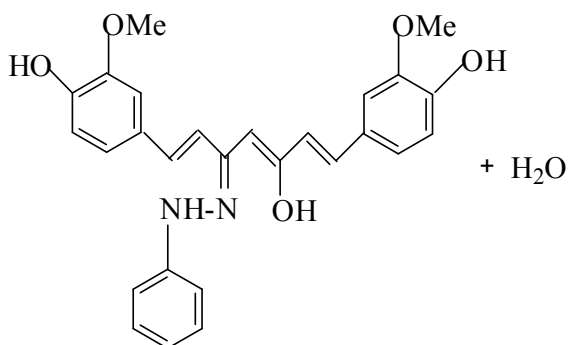
Ethanol  
8 Hrs Reflux  
Cooled



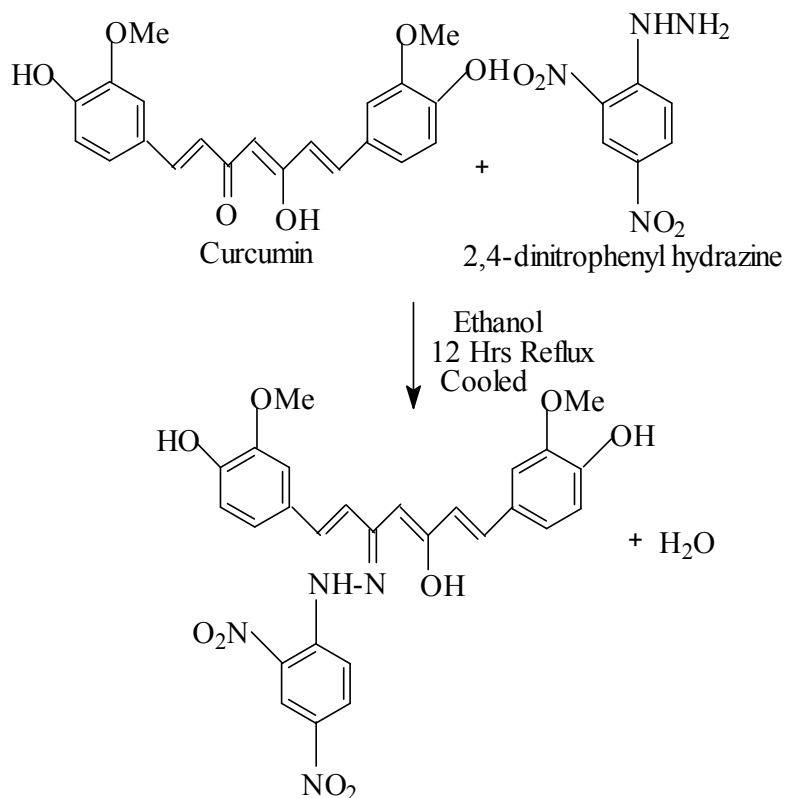
**Compound-I (C-I)**



Ethanol  
4 Hrs Reflux  
Cooled



**Compound II (C-II)**



**Compound – III (C-III)**

The physical characterization and various spectral data of the synthesized compounds C-I, C-II and C-III were tabulated in Table-I.

**TABLE-1 PHYSICAL AND SPECTRAL CHARACTERISATION OF SYNTHESISED CURCUMINOIDS**

S.No	Description	C-I	C-II	C-III
1.	Yield (%)	78	74	82
2.	Empirical formula	C <sub>21</sub> H <sub>21</sub> O <sub>5</sub> N <sub>2</sub>	C <sub>27</sub> H <sub>26</sub> O <sub>5</sub> N <sub>2</sub>	C <sub>21</sub> H <sub>24</sub> O <sub>9</sub> N <sub>4</sub>
3.	M. Wt.	382	458	548
4.	Melting point ( °C)	188	175	201
5.	Colour	Cream	Dark Brown	Dark Brown
6.	pH	9.47	8.94	4.15 z

7.	Found (Calcd) %	C	65.73 (65.97)	70.63 (70.74)	59.08 (59.12)
		H	5.67 (5.76)	5.42 (5.68)	4.28(4.38)
		N	7.49 (7.33)	6.31(6.11)	10.26(10.22)
8.	$\lambda_{\max}$ (nm)		318, 396	333, 430	345, 434
9.	IR data (cm <sup>-1</sup> )		-OH : 3500 – 2700, -NH <sub>2</sub> ; 3300, -OMe : 1375, -C=N : 1593, -C=C- : 1512, aromatic ring -C=C- : 1465, - C - O- C- for OMe : 1225, enolic -C-O- : 968, Phenolic stretch : 1340-1280. Aliphatic -C=CH stretch: 696, 752 and 820.	-OH : 3500 – 2700, -NH; 3240, -OMe : 1375, -C=N : 1593, -C=C- : 1512, aromatic ring -C=C- : 1465, - C - O- C- for OMe : 1225, enolic -C-O- : 968, Phenolic stretch : 1340-1280. Aliphatic -C=CH stretch: 696, 752 and 820.	-OH : 3500 – 2700, , -NH; 3220, -OMe : 1375, -C=N : 1593, -C=C- : 1512, aromatic ring -C=C- : 1465, - C - O- C- for OMe : 1225, enolic -C-O- : 968, Phenolic stretch : 1340-1280. Ar-NO <sub>2</sub> ; 860, Aliphatic -C=CH stretch: 696, 752 and 820.
10.	<sup>1</sup> H NMR data <sup>14</sup> (PPM)		-OMe merged (s) : 4.1, -NH <sub>2</sub> : 3.8, phenolic - OH (s) : 7.2, enolic - OH (s) : 11.4, active -CH (s) : 6.2, -C=CH on enolic side (d,d) 5.4 & 6.4, -C=CH on azomethine side (d,d) : 5.9, 6.7, Benzene ring in curcumin moiety (merged s,d,d) : 7.54, 7.4, 7.6.	-OMe merged (s) : 4.0, -NH- : 5.3, phenolic - OH (s) : 7.4, enolic - OH (s) : 11.2, active -CH (s) : 6.3, -C=CH on enolic side (d,d) 5.7 & 6.1, -C=CH on azomethine side (d,d) : 6.5, 6.7, Benzene ring in curcumin moiety (merged s,d,d) : 6.96, 7.2, 7.32, Benzene ring in phenylhydrazene (merged d,q,q) : 7.5, 7.65 and 7.8.	-OMe merged (s) : 3.91 -NH- : 5.35, phenolic - OH (s) : 7.3, enolic - OH (s) : 11.1, active -CH (s) : 6.45, -C=CH on enolic side (d,d) 5.9 & 6.1, -C=CH on azomethine side (d,d) : 6.4, 6.8, Benzene ring in curcumin moiety (merged s,d,d) : 7.14, 7.2, 7.35, Benzene ring in 2,4-dinitrophenylhydrazene moiety (merged s,d,d) : 7.5, 8.2 and 8.6.

### Anti ulcer Study

The anti-ulcer effects of the investigated compounds were studied by standard procedure.<sup>8&9</sup> Aspirin induced ulcers were treated in Wistar male albino rats by suspending the synthesized compounds in 2 % gum acacia solution. Ranitidine was used as standard drug. Albino rats having 150g-200g were housed five in each group in a standard laboratory conditions. They were fasted for 24 h before doing the experiment and were given water ad libitum. The drugs were administered orally. Group 1 animals received 1ml of gum acacia solution and kept as control. Group 2 animals received Ranitidine (100 mg/kg i.p) Group 3, group 4 group 5 and group 6 animals received C-I, C-II, C- III and C compounds 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.<sup>10</sup> The stomachs were isolated, washed gently under clean flowing water and gastric content was collected, cut open along the greater curvature. The stomachs were then fixed in 10 % formalin and ulcer scores were recorded.<sup>11</sup> 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-azobenzene with phenolphthalein) as indicator. When the colour turned to orange the volume of

sodium hydroxide corresponds to the free acidity. 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 using the formula:

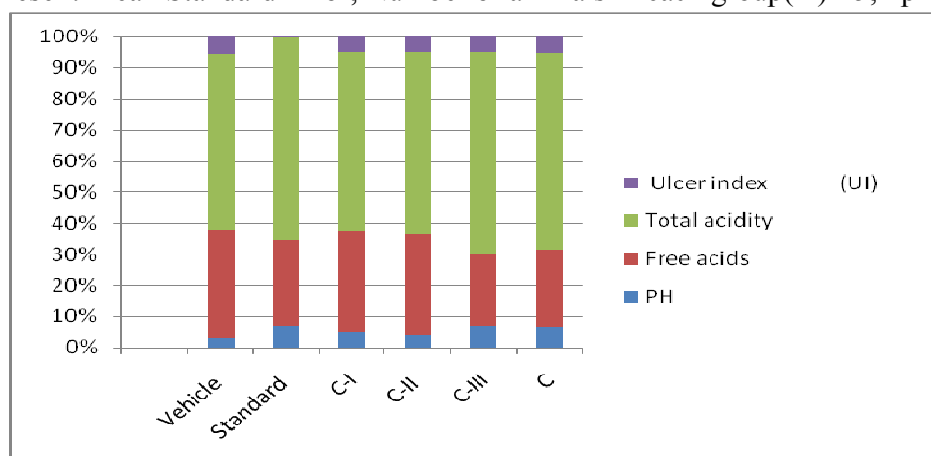
$$\text{Ulcer index} = \frac{10}{\bar{X}} \quad \text{Where, } \bar{X} = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}$$

The data were analyzed by using ANOVA followed by multiple range test and were reported in Table -II.

**Table II. Anti-ulcer effect of synthesized compounds in aspirin induced ulcers 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-I	3.22*±0.19	21.4*±0.62	38.16*±0.26	3.13*±0.16
4	C-II	2.80*±0.02	22.9*±0.34	42*±0.40	3.4*±0.16
5	C-III	3.5*±0.02	11.37*±0.6	32.47±0.40	2.35*±0.17
6	C	3.6*±0.2	12.9*±0.19	33.26*±0.62	2.72*±0.12

± represent Mean Standard Error, Number of animals in each group(n)=5; \*p=<0.001



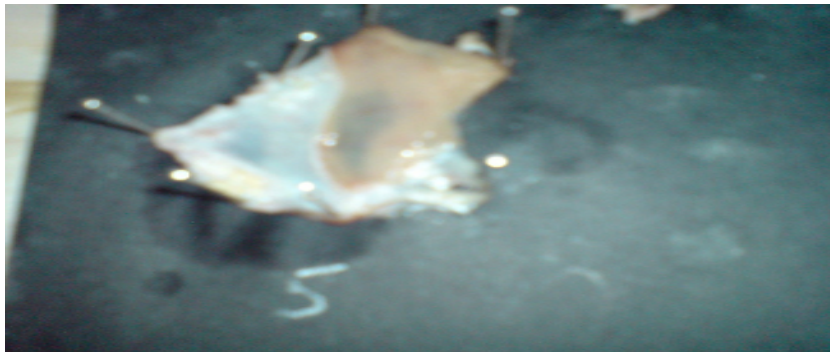


Figure-1 C-I

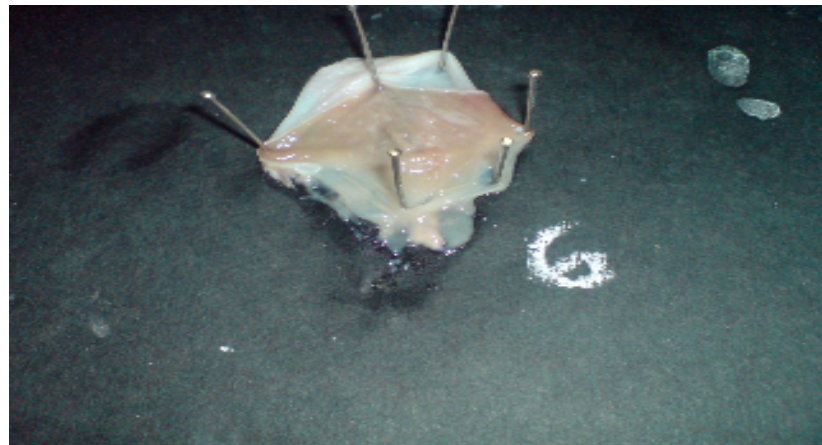


Figure-2 C-II

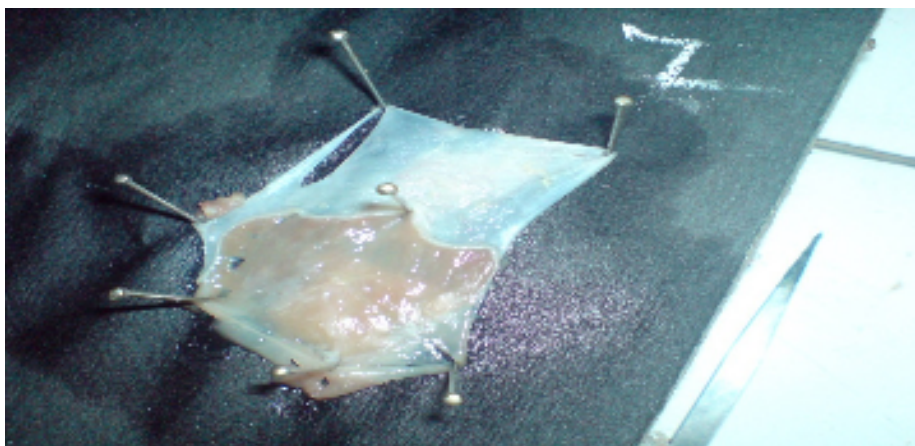


Figure-3 C-III

### Results

The Curcuminoids C-I, C-II and C-III were synthesized and characterized by physical and spectral methods and were mentioned in the table-I. The newly synthesized Curcuminoid III has better effect in healing the ulcer than the parent Curcumin. Compound I has better anti-ulcer effect than the compound II but both are having less effect than Curcumin. The anti-ulcer effect of the synthesized Curcuminoids have shown the following

The pH was observed as C- 3.6, C-I- 3.22, C-II - 2.8, C-III- 3.5.  
The free acid estimated were C- 12.9, C-I- 21.4, C-II - 22.9, C-III-11.37.  
Total acidity observed were C- 33. C-I- 38.16 C-II - 42, C-III-32.47.  
The ulcer Index calculated was C-2.72, C-I-3.13, C-II- 3.4, C-III-2.35.  
The standard drug Ranitidine has the maximum effect in healing the ulcer.

### Discussion

The Curcuminoids were prepared by the condensation reaction. Curcumin possesses anti ulcer effect, but it was not used as a drug for therapeutic treatment. Hence the synthesized drugs were studied for their anti ulcer effect and compared with Curcumin and the standard drug Ranitidine. The study was carried out in aspirin induced ulcers in albino rats by using 2 % gum acacia solution as vehicle and Ranitidine as standard. The result showed that new Curcuminoids have better effects than the parent Curcumin. In addition to this, compound III has greater anti-ulcer effect than the compound II & compound-I. Ulcers are produced when any factor causes an imbalance between the protective factors and aggressive factors in the stomach.<sup>12&13</sup> It protects the gastric mucosa by mechanisms other than gastric acid secretion<sup>14</sup> which include the inhibition of leukotrienes,<sup>15</sup> pepsinogen<sup>16</sup>, free radical scavenging,<sup>17</sup> increasing gastric mucosal blood flow,<sup>18</sup> increasing the protective glycoprotein content and thereby strengthens the gastric mucosa<sup>19</sup> and prevention of oxidation of the mucosal xanthine dehydrogenase<sup>20</sup>. The anti-ulcer activity was displayed by attenuating the different ulcerative effectors including gastric acid hyper-secretion, total peroxides, myeloperoxidase (MPO) activity, IL- 6 and apoptotic incidence.<sup>21</sup> The mucosal layer of stomach was protected from aspirin induced damage by the inhibition of prostaglandin synthesis, which is essential for mucosal integrity and regeneration.<sup>22</sup> This results to a sustained reduction in mucosal blood flow and a subsequent generation of ulcer. The synthesized Curcuminoids have a protective effect against gastric ulcer development. Further studies will be continued to provide a good anti ulcer drug near future.

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