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Bioformulation of an ecofriendly antitumour agent using the herbal extract from *Curcuma aromatica* Salisb. and *Ixora coccinea* L.

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Abstract
This paper represents the antitumours potentialities of the crude rhizomatous extract of *Curcuma aromatica* Salisb. and leaf extract of *Ixora coccinea* L. First, the crude extract from two different plants were taken separately for antitumour screening and then both the extract used simultaneously to evaluate the better efficiency of the herbal formulation. The extract mixture shows higher efficiency in respect to antitumour potentiality than either of the plant when used solely. After that, I performed sequential solvent partitioning of the extract mixture to locate which fraction actually carries the antitumour one. Diethyl ether fraction was found to contain some bioactive phytochemical(s) that can be of ecofriendly use to control the tumorous growth of the biological systems.

Keywords: phytochemicals, antitumorous potentialities, extract mixture, diethyl ether fraction, active principles, *Curcuma aromatica* salisb., *Ixora coccinea* L.

1. Introduction
Zingiberaceae family constitutes a vital group of rhizomatous medicinal and aromatic plants characterised by the presence of volatile oils and oleoresins of export value. Generally, the rhizomes and fruits are aromatic, tonic and stimulant; occasionally they are nutritive. Some are used as food as they contain starch in large quantities while others yield an astringent and diaphoretic juice.

Curcuma aromatica is the vanharidra mentioned in the classical Ayurvedic texts. It is found distributed from China southwards to Sri Lanka. *C. aromatica* holds an important place in native perfumery and cosmetics. It grows wild in many parts of India, and is cultivated in Andhra Pradesh and Orissa. Fresh rhizome is yellowish and gives out a strong camphoraceous smell. Rhizome was valued as medicine, being regarded as a tonic and carminative, and was a valued toiletry and cosmetic item.

In addition to *C. longa*, the genus includes other economically important species such as *C. aromatica*, used in medicine and in toiletry articles; *C. kwangsiensis*, *C. ochrorhiza*, *C. pierreana*, *C.zedoaria*, *C. caesia*, etc., used in folk medicines of the Southeast Asian nations; *C. alismatifolia* etc., having floricultural importance; *C. amada*, used as a vegetable in a variety of culinary preparations, pickles, and salads; and *C. zedoaria, C. malabarica, C. pseudomontana, C. montana, C. decipiens, C. angustifolia, C. aeruginosa*, etc., used in the production of arrowroot powder [1].

Ixora coccinea L. belongs to the family Rubiacae, is a common flowering shrub native to Asia including Bangladesh, Southern India, and Sri Lanka [2]. The flowers of *I. coccinea* are used in the treatment of dysentery, leucorrhoea, dysmenorrheal, haemoptysis, bronchitis and scabies [3]. The antimicrobial properties of the flower [4] and leaves [5] have also been reported.

Present study involves the evaluation of antitumour potentialities using the extract mixture of *Curcuma aromatica* and *Ixora coccinea*. This study also incorporates some additional evaluation of this mixture as an ecofriendly cytotoxic agent that can be applied in living biological systems.

2. Materials and Methods
2.1 Preparation of *Curcuma aromatica* rhizomatous extract
2.5 kg shade dried rhizomes of *Curcuma aromatica* Roxb. plants was powdered and extracted three times with 95% EtOH (each 500 ml, 48 h) at room temperature. The extract was evaporated under reduced pressure and a solid residual mass was obtained. The residual solid obtained was then subjected to sequential solvent partitioning for locating the antitumorous property of the plant. As the crude extract was positive in antitumour assay, sequential solvent partitioning of the crude rhizomatous extract of *Curcuma aromatica* and identification of the antitumorous fraction was performed.

For the sake of convenience the fraction obtained from *Curcuma aromatica* was assumed as residue A.

2.2 Preparation of *Ixora coccinea* leaf extract:
2.5 kg shade dried leaves of *Ixora coccinea* were ground to a fine powder and then extracted in 5 liter of 50% aqueous ethanol at room temperature for 7 days. The extract was filtered and concentrated under reduced pressure and a solid, dark brown residual solid was obtained. The residual solid obtained was then subjected to sequential solvent partitioning for locating the antitumours property of the plant. As the crude extract was positive in antitumour assay, sequential solvent partitioning of the crude leaf extract of *Ixora coccinea* and identification of the antitumours fraction was performed.

For the sake of convenience the fraction obtained from *Ixora coccinea* was assumed as residue B.

2.3 Sequential solvent partitioning of residue A and residue B separately followed by identification of the antitumour fractions

Both the extract was filtered and the filtrate was charcoalised separately. The charcoalised fraction was separatey filtered repeatedly through Whatman No.42 filter paper and a clear brown filtrate was obtained. Two different filtrates were then separately partitioned over petroleum-ether (60-80ºc), diethyl ether and chloroform in two different sets. Each fraction was collected separately, dried over anhydrous sodium sulphate and was concentrated under reduced pressure. A brown residual solid mass was obtained in each case from residue A and B. Both the residual mass obtained from *C. aromatica* and *I. coccinea* was again diluted separately in different container to produce different concentrations (125 mg ml^{-1} to 1 mg ml^{-1}) and their antitumour property was evaluated.

Furthermore five different dilution sets were prepared where 50% of the residual solid mass taken from *C. aromatica* and rest 50% (in mass/mass ratio) residual solid mass taken from *I. coccinea.* Here also the mixture was diluted in different concentrations (125 mg ml^{-1} to 1 mg ml^{-1}) followed by evaluation of their antitumorous property.

In each of the experiments a control set was maintained and LC_{50} values were determined.

2.4 Preparation of sample solution

The test solution was prepared by dissolving the dark brown residual mass in few drops of propylene glycol and then diluting with sterile water [6] in the concentration of 125 mg ml^{-1} to 1 mg ml^{-1}. Few drops of propylene glycol diluted with sterile water were used as control. All the dilutions were sterilized by filtration using membrane filter (0.02 µ pore size).

2.5 Antitumorous assay

Brine Shrimp Cytotoxicity [7, 8] assay was performed with two different kinds of extracts separately and their mixture. Brine shrimp eggs were hatched in a shallow rectangular dish (22×32×12 cm), one third of which was filled with saline water. An aluminium divider with several 2 mm holes was clamped in the dish to make to unequal compartments. The eggs (50 mg) were sprinkled into the larger compartment which was darkened while the smaller compartment was illuminated. The set was maintained at 30^0C- 32^0C and after 48 hours the phototropic nauplii was collected by pipette from the lighter side, having been separated by the divider from their shells.

The shrimps were transferred to each sample(s) vial using a 23 cm disposable pipette and saline water was added to adjust the volume to 5 ml. The nauplii could be counted in the stem of the pipette against a lighted background. A drop of dry yeast suspension (3 mg in 5 ml of saline water) was added as food to each vial. The vials were maintained under illumination at room temperature. Surviving shrimps was counted after every 3 hours up to 24 hours and the percentage of death at each dose and control was determined.

$$\text{Death (\%)} = \text{test-control} \times 100/\text{control}$$

Four replicates was prepared for each dose level and LC_{50} values were determined.

2.6 Statistical analysis

The observed values were expressed as mean ± standard deviation. All the values reflected in the table are statistically analyzed by paired t test with control and found significantly different at 5% level ($P<0.05$)

3. Results

3.1 Antitumorous activity screening of the crude extract of *Curcuma aromatica*

The LC_{50} value of this sample was 25 mg/ml. **(Table 1).** Death of shrimp increases with the increase concentration of crude extract.

3.2 Antitumorous activity screening of the crude extract of *Ixora coccinea*

The LC_{50} value of this sample was 50 mg/ml. **(Table 2).** Death of shrimp increases with the increase concentration of crude extract.

3.3 Antitumorous activity screening of the extract mixture

Interestingly, the LC_{50} value of this sample was 10 mg/ml. (Table 3). Death of shrimp increases with the increase concentration of crude extract.

Table 1: Determination of antitumour activity of the crude extract of *Curcuma aromatic*

Conc. (mg/ml)	No. of survivals after									LC_{50}
	0 hours	3 hours	6 hours	9 hours	12 hours	15 hours	18 hours	21 hours	24 hours	
125	18.0 ±0.0	13.4 ±0.5*	10.2 ±0.4*	8.4 ±0.5*	6.6 ±0.5*	4.8 ±0.4*	3.2 ±0.4*	2.8 ±0.4*	2.6 ±0.5*	
100	18.0 ±0.0	15.2 ±0.4*	13.2 ±0.4*	11.4 ±0.5*	8.2 ±0.4*	7.6 ±0.5*	7.4 ±0.5*	5.2 ±0.4*	3.2 ±0.4*	
75	18.0 ±0.0	18.0 ±0.1*	16.2 ±0.4*	15.2 ±0.4*	12.6 ±0.5*	9.6 ±0.5*	7.8 ±0.4*	5.6 ±0.5*	4.2 ±0.4*	
50	18.0 ±0.0	18.0 ±0.0	16.8 ±0.4*	16.8 ±0.4*	15.8 ±0.4*	13.6 ±0.5*	9.8 ±0.4*	8.2 ±0.4*	7.2 ±0.4*	
25	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	17.8 ±0.4*	16.8 ±0.4*	13.0 ±0.7*	10.8 ±0.4*	9.6 ±0.5*	9.0 ±0.7*	25 mg/ml
10	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	17.8 ±0.4*	17.6 ±0.5*	17.2 ±0.4*	15.6 ±0.9*	12.8 ±0.8*	
1	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	17.8 ±0.4*	17.8 ±0.4*	17.2 ±0.4*	16.8 ±0.4*	
0 (Control)	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	

The observed values were expressed as mean ± standard deviation.

Calculation was done with the help of spread sheet software Microsoft Excel 2010.

* Indicates significance at ($P<0.05$)

Table 2: Determination of antitumour activity of the crude extract of *Ixora coccinea*

Conc. (mg/ml)	No. of survivals after									LC_{50}
	0 hours	3 hours	6 hours	9 hours	12 hours	15 hours	18 hours	21 hours	24 hours	
125	18.0 ±0.0	13.4 ±0.5*	10.2 ±0.4*	8.4 ±0.5*	6.6 ±0.5*	4.8 ±0.4*	3.2 ±0.4*	2.8 ±0.4*	2.6 ±0.5*	
100	18.0 ±0.0	15.2 ±0.4*	13.2 ±0.4*	11.4 ±0.5*	8.2 ±0.4*	7.6 ±0.5*	7.4 ±0.5*	5.2 ±0.4*	3.2 ±0.4*	
75	18.0 ±0.0	18.0 ±0.1*	16.2 ±0.4*	15.2 ±0.4*	12.6 ±0.5*	9.6 ±0.5*	7.8 ±0.4*	5.6 ±0.5*	4.2 ±0.4*	
50	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	17.8 ±0.4*	16.8 ±0.4*	13.0 ±0.7*	10.8 ±0.4*	9.6 ±0.5*	9.0 ±0.7*	50 mg/ml
25	18.0 ±0.0	18.0 ±0.0	16.8 ±0.4*	16.8 ±0.4*	15.8 ±0.4*	13.6 ±0.5*	9.8 ±0.4*	8.2 ±0.4*	7.2 ±0.4*	
10	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	17.8 ±0.4*	17.6 ±0.5*	17.2 ±0.4*	15.6 ±0.9*	12.8 ±0.8*	
1	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	17.8 ±0.4*	17.8 ±0.4*	17.2 ±0.4*	16.8 ±0.4*	
0 (Control)	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	

The observed values were expressed as mean ± standard deviation.
Calculation was done with the help of spread sheet software Microsoft Excel 2010.
* Indicates significance at ($P<0.05$)

Table 3: Determination of antitumour activity of the extract mixture

Conc. (mg/ml)	No. of survivals after									LC_{50}
	0 hours	3 hours	6 hours	9 hours	12 hours	15 hours	18 hours	21 hours	24 hours	
125	18.0 ±0.0	13.4 ±0.5*	10.2 ±0.4*	8.4 ±0.5*	6.6 ±0.5*	4.8 ±0.4*	3.2 ±0.4*	2.8 ±0.4*	2.6 ±0.5*	
100	18.0 ±0.0	15.2 ±0.4*	13.2 ±0.4*	11.4 ±0.5*	8.2 ±0.4*	7.6 ±0.5*	7.4 ±0.5*	5.2 ±0.4*	3.2 ±0.4*	
75	18.0 ±0.0	18.0 ±0.1*	16.2 ±0.4*	15.2 ±0.4*	12.6 ±0.5*	9.6 ±0.5*	7.8 ±0.4*	5.6 ±0.5*	4.2 ±0.4*	
50	18.0 ±0.0	18.0 ±0.0	16.8 ±0.4*	16.8 ±0.4*	15.8 ±0.4*	13.6 ±0.5*	9.8 ±0.4*	8.2 ±0.4*	7.2 ±0.4*	
25	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	17.8 ±0.4*	17.6 ±0.5*	17.2 ±0.4*	15.6 ±0.9*	12.8 ±0.8*	
10	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	17.8 ±0.4*	16.8 ±0.4*	13.0 ±0.7*	10.8 ±0.4*	9.6 ±0.5*	9.0 ±0.7*	10 mg/ml
1	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	17.8 ±0.4*	17.8 ±0.4*	17.2 ±0.4*	16.8 ±0.4*	
0 (Control)	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	18.0 ±0.0	

The observed values were expressed as mean ± standard deviation.
Calculation was done with the help of spread sheet software Microsoft Excel 2010.
* Indicates significance at ($P<0.05$)

4. Discussion

From the above results it can be conclude that, the diethyl ether fraction of the extract mixture showed better antitumorous potentiality over the extracts of *Curcuma aromatica* or *Ixora coccinea*. At a minimal of dilution of the extract mixture, the LC_{50} value of this sample was 10 mg/ml, which is far more effective than the sole effect of the extract of *Curcuma aromatica* or *Ixora coccinea*. Table 3 also reflects that the dose becomes very much lethal to the shrimp by the extract mixture even the lethality caused by any extract alone at the same concentrations. Hence, the extract mixture can be identified as a better ecofriendly bioformulation of antitumour compounds.

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6. References

1. Ravindran PN, Babu KN, Sivaraman K. Termeric- The genus *Curcuma*, CRC press- Taylor & Francis group, Boca Raton, London, New York, 2007.
2. Ghani A. Medicinal plants of Bangladesh with chemical constituents and uses. Dhaka, The Asiatic Society of Bangladesh. 2003, 267.
3. Anonymous, The wealth of India, A Dictionary of Indian Raw materials and Industrial Products, Council of Scientific and Industrial Research, New Delhi. 1959, 91.
4. Latha PG, Abraham TK, Panikkar KR. Antimicrobial properties of *Ixora coccinea* L. Ancient Sci. Life. 1995; 14:286-290.
5. Annapurna J, Amarnath PVS, Amar DK, Ramakrishna SV, Raghavan KV. Antimicrobial activity of *Ixora coccinea* leaves, Fitoterapia. 2003; 74:291-293.
6. Mukherjee PK, Saha K, Giri SN, Pal M, Saha BP. Antifungal screening of *Nelumbo nucifera* (Nymphaeaceae) rhizome extract, Ind. J. Microbiol. 1995; 35:327.
7. Meyer BN, Ferrigni NR, Putnam JE, Jacobson LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plants constituents, Planta Med. 1982; 45:31-34.
8. Ramalakshmi S, Edaydulla N, Ramesh P. Investigation on cytotoxic, antioxidant, antimicrobial and volatile profile of *Wrightia tinctoria* (Roxb.) R. Br. flower used in Indian medicine, Asian Pac. J. Trop. Dis, 2012, D 68 - S 75.