

Research Article**Cytotoxicity activity of *Erythrina indica* flowers and separation of essential oil**

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Abstract

Objective: To study the anticancer potential and separation of essential oil from flowers of *Erythrina indica*. **Material and methods:** Antibacterial efficiency was checked by well diffusion method. In to the prepared Muller Hinton agar plates, test cultures were swabbed, incubated for 24 hours. Then well was made and added different concentrations of extract. Cytotoxicity test was done by adding various concentration of extract to the cell lines, incubated and finally MTT solution was added. Formazan formation was measured at 550nm. Cell morphology was checked by microscopy analysis and steam distillation was carried out to separate essential oil. **Results and conclusion:** Among the 5 test organism analysed, extract showed maximum zone of inhibition for *S. aureus*. It was identified that the flower ethanol extract was effective against both the cell lines with the cytotoxicity % of 71.26 towards HepG2 and against MG-63 showed 67.11. Hydrosol and the essential oil have to check for its chemical composition, pharmaceutical applications.

Keywords: *Erythrina indica*, antibacterial, HepG2, hydrosol

Introduction

Globally utilization of plants as therapeutics agent is increasing. At the same time when a plant is located as valuable drug, its population become prone to wild crafting and unsustainability. Considering this WHO (World health organisation, 2003) and European Medicines Agency (EMA, 2006) developed guidelines for collection and sustainability of medicinal plants (Atanasov et al., 2015). Vegetarian foods are considered as super nutrient source, as it contains flavanoids, alkaloids and other pharmaceutically important compounds (Shakya, 2016). In this study, cytotoxicity potential of and essential oil extraction of *Erythrina indica* (EI) flower was carried out to explore its medicinal importance.

Erythrina indica is a fabaceae plant commonly found in tropical and subtropical regions of world. This plant can grow up to a height of 30- 60 meters. It can be referred as coral tree commonly as it holds red colour flower. These are coral trees peculiarly cultivated for their ornamental flowers, they play

major role in nitrogen fixation in soil. The plant is commonly known as 'Kalyana murungai' in Tamil language (Lahari et al., 2015). Flowering is continued by seed production and they are brown in colour (Nagar and Chauhan, 2015).

IARC, 2018 (12th September) release states that new cancer affected cases reached 18.1 million and 9.6 million deaths. Also it was identified that the lung cancer, female breast cancer and colorectal cancer are more prevalent. In men, the leading cause for the death is lung cancer which followed by prostate cancer, colorectal, liver and stomach cancer. Likewise in women the breast cancer is the most commonly diagnosed, followed by lung, colorectal and cervical cancer (www.iarc.fr/en/media-centre/pr/2018/pdfs/pr263_E.pdf).

Liver cancer is recorded as third leading cause of cancer associated death. Liver trans-plantation, surgical resection and chemotherapy are the common treatment practice of liver cancer. But the chemotherapeutic agents available are not effective in advanced stage of cancer and so the plant based drugs which can work in such case will be boon to the society (Jiao et al., 2018). Mostly children and young adults are affected by osteosarcoma. Like in other cases of cancer, bone cancer also treated surgical or by chemotherapy (Jirangkul et al., 2014). As there is a large need of plant based agent to treat cancer, the present study is focussed on assessing the anticancer potential of EI flower.

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Materials and methods

Ethanol extraction of *Erythrina indica* (EEI)

Fresh flowers of *Erythrina indica* was collected from local place of Chennai city washed and dried in shade for 1 week and authenticated. Shade drying is the traditional method commonly followed for drying the plant material (Dwivedy et al., 2012). 10g of EI flower was transferred to the conical flask containing 100ml of ethanol, placed in orbital shaker with for 7 days, filtered, evaporated and stored (Okoduwa et al., 2016).

Metabolite analysis

Preliminary analysis of secondary metabolites was carried out by Trease and Evans procedure (Trease and Evans, 2009).

Antibacterial activity

Culture plates were made by pouring Muller Hinton agar to the depth of 3-4mm, allowed to dry. Then the test culture (*E. coli*, *A. hydrophilia*, *K. pneumonia*, *B. cereus* and *S. aureus*) was swabbed evenly on to the agar and incubated for 24hrs at 37°C. Wells were made of 5mm diameter. 30 µl of three different concentrations (10µg/ml, 20µg/ml and 30µg/ml) of EEI and ampicillin were added to pre-marked wells. After 24 hrs incubation the zone of inhibition was measured (Yadav et al., 2015).

Cell culturing and cytotoxicity assay

Cell lines were properly cultured in DMEM (Dulbecco's modified eagle medium) and grown overnight (96- well plate) at 37°C in a 5% CO₂ incubator. EEI of five different concentration (20, 40, 60, 80 and 100 µg/ml) were added, incubated for 24hrs. Then 50 µL of 2 mg/mL MTT solution was added to the well and incubated for 4hrs. After incubation, all supernatant was discarded and 100 µL of dimethyl sulfoxide was added to dissolve the formazan crystals. Absorbance was measured at 550nm and cell viability was calculated. MTT is cleaved by mitochondria dehydrogenase in viable cells, giving a measurable purple product formazan. This formazan production is directly proportionate to the viable cell number and inversely proportional to the degree of cytotoxicity (Han et al., 2015). Morphological change was observed under inverted microscope (Husni et al., 2015).

Steam distillation

Essential oil was extracted by steam distillation procedure. Essential oil has applications in pharma, agricultural, food and perfume industries (Gakuubi, 2016).

Results and discussion

Alkaloids and phenolic compounds like tannins, flavanoids were showed positive in EEI (Table 1). Different solvents extract of EI leaf revealed the same kind of secondary metabolites as in EEI (Kumari et al., 2017).

Table 1. Secondary metabolites of EEI

S. No	Test for bioactive compounds	<i>Erythrina indica</i> ethanol extract
1	Alkaloids test	+
2	Flavonoids test	+
3	Saponins test	-
4	Phenolic compound test	+
5	Steroids test	-
6	Tannins test	+
7	Terpenoids	+

+ positive, - negative.

EEI was found to be highly effective against *S. aureus* with 6.1mm as maximum zone of inhibition, whereas against *K. pneumoniae* it showed 5.4mm, *E.coli* of 5.1mm and *B.cereus* with 2.6mm. *A. hydrophilia* showed the lowest zone of inhibition (Figure 1, 2, 3). Stem methanolic extract of EI was found to be effective against *E. coli* (18mm)



Figure 1. Zone of inhibition of *E. coli*, *A. hydrophilia*

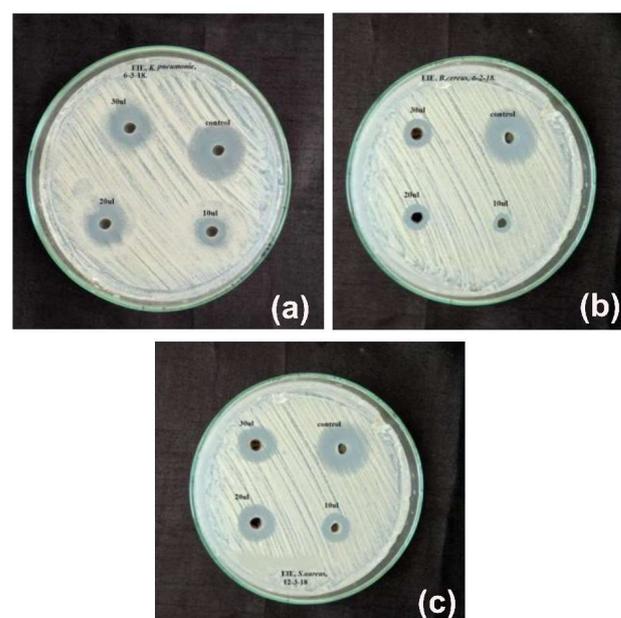


Figure 2. Zone of inhibition of (a) *K. pneumoniae* (b) *B. cereus* (c) *S. aureus*

comparable to *S. aureus* (10mm) (Sahoo et al., 2012). Similarly aqueous leaf extracts was also have high inhibitory activity against *E. coli* than towards *S. aureus* as in case of methanol extract of EI stem (Kumari et al., 2017). Ethanol leaf analysed by Christy Jeyaseelan et al. (2017) revealed that the extract has potential inhibition against *E. coli* but *S. aureus* showed less inhibition potential (Jeyaseelan et al., 2017).

Cytotoxicity potential was dose dependent against HepG2 and

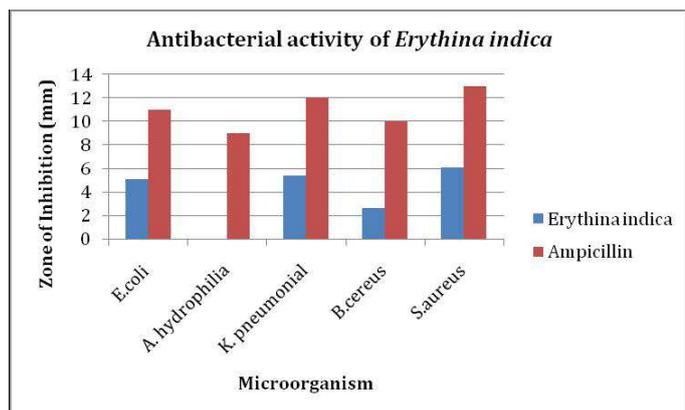


Figure 3. Antibacterial activity of *Erythrina indica*

MG63. EEI showed 71.26% of cytotoxicity against HepG2 whereas 67.11% cytotoxicity towards MG63 (Table 2). Thorough literature review reveals that the leaves and the other parts of EI has checked for anticancer potential. EI leaf ethanol extract against adenocarcinoma results showed 78.89% cytotoxicity at 1000 μ g/ml (Priya et al., 2017). 10, 11-dioxoerythratidine and the crystagallin A were the two main compounds of EI leaf and stem showed potential anticancer activity against breast cancer T47D cell- line (Herlina et al., 2011). Morphology analysis revealed that EEI presence caused cell shrinkage which was not seen in control (Figure 4a, b, c, d). Breast cancer cell line assessed with aqueous *Erythrina indica* extract also showed similar morphological change when studied with acridine orange and Giemsa stains (Rai et al., 2017). Further analysis of pure compound responsible for anticancer activity is needed.

Essential oil extracted was separated from hydrosol by freezing method. 1ml of essential oil was collected. Both hydrosol and essential oil have wide application and so the constituents of the obtained oil, hydrosol has to be studied in detail in future. Essential oils are widely utilized because of its limited side effects (Mahmoudi, 2017). Hydrosols of

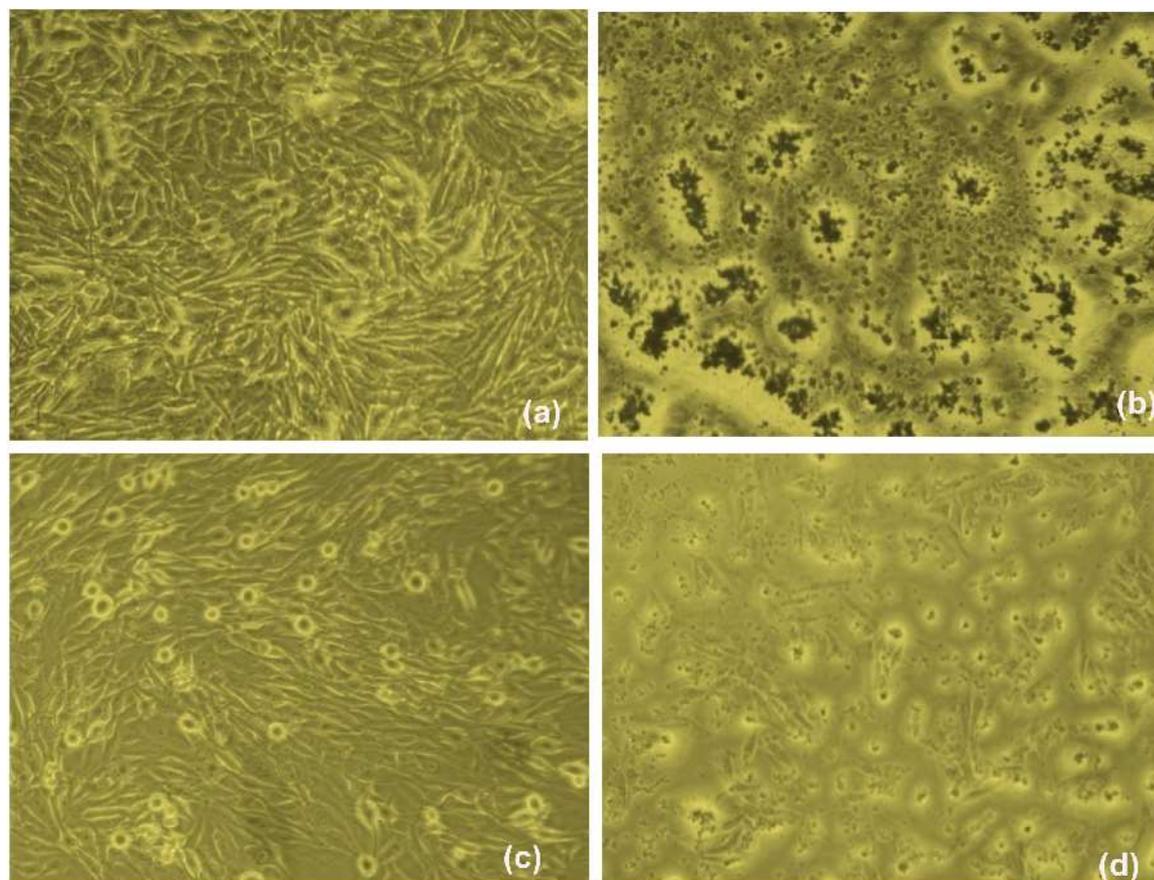


Figure 4. Cytotoxicity effect of (a) Control (HepG2); (b) EEI (HepG2); (c) Control (MG-63); (d) Cytotoxicity of EEI (MG-63)

Table 2. Cell toxicity assay

Concentrations (µg/mL)	Cell toxicity (%) HepG2	Cell toxicity (%) MG-63
Control	0	0
20	11.34	8.86
40	26.75	19.87
60	53.04	43.32
80	61.23	58.97
100	71.26	67.11

Lavender showed efficient antimicrobial activity as well as a potential preservative in moisturizing body gel (Styczynska et al., 2014).

Conclusion

Erythrina indica flower is commonly known for its ornamental purpose but its medicinal value observed showed that it can be utilized for drug development by carrying out in-vivo study and also pure bioactive compound isolation can be done for effective results.

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Conflict of interest

There is no conflict of interest.

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