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Antitumor and Antioxidant Potential of Ethyl Acetate Extract of *Ixora Singaporensis* Hort Against Ehrlich Ascites Carcinoma Tumor

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Abstract
Ixora singaporensis leaves have been traditionally used for many ailments including cancer. In the present study, anticancer activity of ethyl acetate extract of *Ixora singaporensis* leaves (EAIS) was evaluated by using in *vitro* and *in vivo* methods. Total flavonoid content and total antioxidant capacity of the extract were determined. EAIS was found antiproliferative against the HeLa cells with the IC50 values recorded at 26 ± 0.6 µg/ml. With increase in concentration of extract (EAIS) 200, 300 and 500 mg, 18.6, 32.56 and 55.81 days increase in life span observed, respectively. Percentage of cell viability also decreases with increase in concentration of extracts. The potency of the extract was compared with standard 5- fluorouracil (10 mg/kg i.p.).

Keywords
Ixora singaporensis (L) hort; Ehrlich ascites carcinoma tumor; Antitumor; HeLa cell lines.

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1. INTRODUCTION
Ixora genus from the family *Rubiacae* comprises around 500 species where names of the plants differ on the basis of colors of the flowers.[1] Ixora plant and its various parts like flowers, leaves, roots, barks and fruits have been used by various ethnic groups of Africa, Asia and Europe. [2-3]
In Ayurveda and ancient Asian medicine, the leaves of these plants are reported for the antiinflammatory, anti-diarrheal, antiasthmatic, hypotensive, antiulcer, antinociceptic, antiviral, antigestagenic and anticancer activity.[4]
Leaves are also used in skin diseases, colic, flatulence, diarrhea, indigestion, ulcers, wounds, headache and stomachache. Flowers are used for amenorrhea, hypertension, whooping cough, ulcers, dysentery, haemoptysis and cancer. They also possess estrogenic and abortifacient properties.[5-7]
The root, leaves, barks are used for the medicinal purpose. Various pharmacological studies on this plant have revealed the antityrosinase [8] and antioxidant [8-9], anthelmintic [10], antileishmania [11], antiasthmatic [12], hypoglycaemic and hypolipidaemic [13], cardioprotective [14], anti-ulcer [15-16], neuroprotective activity [17], anxiolytic [18].
Ixora singaporensis, belongs to the family *Rubiacae*, is a common flowering shrub native to Singapore, as the name suggests. It is also present in Asia including Bangladesh, Southern India, and SriLanka [2-3].
The preliminary phytochemical screening of methanol, ethyl acetate and chloroform extracts of leaves of *Ixora singaporensis* confirms the presence of flavonoids, phenol, alkaloids, tannin, steroids,

triterpenes, anthraquinones and saponins etc. The weight of the epidemiological evidence for a protective effect of flavonoids against cancer is impressive. A growing number of epidemiological studies suggest that high flavonoid intake may be correlated with a decreased risk of cancer. Flavonoids may inhibit various stages in the carcinogenesis process, namely tumor initiation, promotion, and progression. The mechanisms of cell death include carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, free radical scavenging and reversal of multidrug resistance or a combination of these mechanisms. Based on this information about flavanoids as anti cancer agents, the present study focus on the anti cancer activity (*in vitro and in vivo*) of the ethyl aetate extract of *Ixora singaporensis*

2. Materials and methods

2.1. Collection of plant material

Plant material: Leaves of *I.* singaporensis were collected from local area of Pune, India in December 2012, and identified by Botanical Survey of India, Pune. A voucher specimen (VIVIIS-1) for this collection has been retained in pharmacognosy department of Govt College of Pharmacy, Ratnagiri (India).

2.2. Tumor cell lines

HeLa and EAC, cell lines were purchased from National center for cell science, Pune. They were maintained by weekly intraperitoneal inoculation of 10^6 cells/ mouse.

2.3. Animals

Male Swiss albino mice weighing 20-25 g were used in this experiment. They were housed in standard environmental condition like, ambient temperature (25°C ±1°C), relative humidity (55±5%), and 12/12h light dark cycle. Animals had free access to standard pellet diet and water ad libitum. All animal experiments were carried out in accordance with the guidelines of CPCSEA. The Institutional Animal Ethical Committee has given the approval for conducting animal experiments (Approval No. 1036/a/07/CPCSEA/IAEC/12-13/D-5)

2.4. Acute oral toxicity study

The oral acute toxicity study was carried out using OECD guidelines 425. The single dose toxicity test was conducted for 14 days. Swiss albino mice (20-25g) were used for acute toxicity study. The overnight fasted mice were divided into 3 mice each of 2 groups. Group 1- normal control animals with only vehicle 10 ml/kg. Group 2- Ethyl acetate extract of *I. singaporensis* leaves with dose of 2000 mg/kg was administered orally. The animals were observed for toxic symptoms and mortality continuously for first 4 hrs after the administration. Finally, numbers of survivors were noted after 24 hrs and these animals were maintained for further 13 days with daily observation of changes in skin and fur eyes and mucous membranes behavior pattern salivation, diarrhea, lethargy, sleep [19].

2.5. Extraction from the leaves of I. singaporensis:

The shade-dried leaves were coarsely powdered and extracted successively with chloroform, ethyl acetate, methanol by soxhlet apparatus. The solvent was completely removed by rotary evaporator and kept in desiccator to obtain gummy exudates. This crude extract was used for further investigation for potential antioxidant properties. Phytochemical screening of the extract revealed the presence of tannin, flavonoids, phenol, alkaloids, steroids, triterpenes and saponins etc.

2.6. Preliminary Phytochemical screening:

The freshly prepared extract of *I. singaporensis* was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the standard procedure [20].

2.7. Determination of total antioxidant capacity

The antioxidant activity of extract was determined by the phosphomolybdenum method. The 0.3 ml of extract was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 4 mM ammonium molybdate, 28mM sodium phosphate). The reaction mixture was incubated at 95^0 C for 90 min and cooled at room temperature. Absorbance of solution was measured at 695 nm using spectrophotometer against blank. Ascorbic acid was used as standard (1mg/ml in distilled water); the total antioxidant capacity was expressed as the number of equivalents of ascorbic acid (AAS) [21].

2.8. Determination of total flavonoids

Test sample (1ml) was taken in the test tube and 4 ml of water was added. 0.3 ml of sodium nitrate, 0.3 m of aluminum chloride were added. Solutions was incubated for 6 min at room temperature, and then 2 ml of NaOH was added to the reaction mixture, made the volume up to 10 ml by adding distilled water. The absorbance of reaction mixture was measured at 510 nm against blank by using spectrophotometer. Quercetin was used as standard (mg/ml in distilled water). Total flavonoids were expressed as Quercetin equivalent in milligrams [22].

2.9. In vitro anticancer activity

2.9.1. MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl teterazolium bromide) assay

Cells were grown in RPMI-1640 medium at 37º C incubation for 6-7 hrs. 5 % CO_2 in a humidified

incubator. Cells were harvested, counted (3×10^4cells/ml), and transferred into a 24 well plate, and incubated for 24hrs, prior to the addition of test compound. Serial dilutions of test samples were prepared by dissolving compounds in DMSO followed by dilution with RPMI-1640 medium to give final concentration at 250, 500, 1000 µg /ml. Stock solutions of samples were prepared. Sample at 10 µl and cell lines at 90 µl were incubated for 72 hrs. MTT solution at 5mg/ml was dissolved in 1ml of phosphate buffer solution (PBS), and 10µl of it was added to each of the 24 wells. The wells were wrapped with aluminum foil and incubated at 37º C for 4 hrs. The solution in each well containing media, unbound MTT and dead cells were removed by suction and 150 µl of DMSO was added to each well. Then the plants were shaken, and optical density was recorded using a microplate reader (spectrophotometer) at 595 nm. DMSO as a blank. Controls and samples were assayed and replicated for each concentration and replicated three times for each cell line. After 24 h incubation of the mononuclear cells with plant extracts, the cytotoxicity on the cancer cell lines was evaluated using MTT assay. The cytotoxicity was obtained by comparing the absorbance of the sample and control solutions. The values were then used to calculate the concentration of plant extracts required to cause a 50 % reduction (IC50) [23].

Cell viability (%) = Mean OD/Control OD x 100

***2.10. In vivo* anticancer activity**

2.10.1. Determination of anti-tumor activity

The animals were acclimatized as per laboratory conditions. They were divided into six groups *viz*. Normal control (G1), Cancer induced control (G2), cancer induced animal treated with 10mg/kg of 5-fluorouracil treated group (G3), 200mg/kg, 300mg/kg, 500mg/kg of EAIS leaves (G4), (G5) and (G6) of ten each and used for the study. The EAC cells were procured from National Center for Cell Science, Pune and injected intraperitonially (2×10^6 cells/mice) to all groups of animals excluding G1 group. On the second day the animals of G4, G5 and G6 were treated with 200, 300 and 500 mg/kg/day p.o. EAIS extract, while G3 with (10 mg/kg. i.p.) and the treatment was continued for next 14 days. G2 was not allocated any treatment after inoculation with EAC cells. The mice were observed for next 14 days for the development of ascitic tumor. On day 15, twenty-four hours of last dose and 18 h of fasting, 6 animals of each group were sacrificed by cervical dislocation to measure antitumor and hematological parameters and the rest were kept with food and water ad libitum to check percentage increase in life span of the tumor host.

The antitumor activity of the extract was measured in EAC animals with respect to the following parameters: Tumor volume, tumor weight, Packed cell volume (PCV), Viable cell count, nonviable cell count, percentage of viable cells, percentage of non viable cells, increase in life span (ILS), average increase in body weight, changes in food intake [24].

2.10.2. Tumor volume

The ascitic fluid from mice was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1000 rpm for 5 min [25].

2.10.3. Tumor cell count

The ascitic fluid was taken in a WBC pipette. After dilution 100 x , a drop of the diluted cell suspension was observed in Neubauer counting chamber [25].

2.10.4. Viability assay (Tryphan blue dye assay)

The cells were then stained with tryphan blue dye. The stained cells were dead cells or nonviable cells. Cytotoxicity was assessed by incubating 1 X 10^6 EAC cells in 1 ml phosphate buffer saline with varying concentration (50-1000 µg/ml) of the extract at 37º C for 3 hours in CO_2 atmosphere. The viability of cells was determined by tryphan blue dye whereas visible cells exclude the dye [26].

2.10.5. Percentage increase life span (% ILS)

Animals were inoculated 1×10^6 cells/ mL 0.1 mL of EAC cells per 10g body weight of the animals was injected (i.p.) on day zero (day 0). A day of incubation was allowed for multiplication of the cells. Fourteen doses of the Test samples (200 mg ,300, 500 mg/kg, 0.1 mL/10g body weight) and control group was treated with same volume of saline (0.9% sodium chloride solution) and compared with 5-Fluorouracil (10 mg/kg body weight) were injected i.p. from the first day up to the 9th day with 24 hrs intervals. The effect of test samples on tumor growth was monitored by recording the mortality daily for a period of 9 days and percentage increase in life span (%ILS) was calculated by following equation. [25].

$$\text{Increase in life span} = \frac{T-C}{C} \times 100$$

2.10.6. Body weight

Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period.

2.10.7. Hematological Parameters

At the end of the experimental period, all mice were sacrificed by cervical dislocation. Blood was collected from freely flowing tail vein and used for the estimation. Hemoglobin (Hb) content, red blood cell count (RBC) [27] and white blood cell count (WBC) [28]. WBC differential count was carried out from Leishman stained blood smears [29].

2.10.8. Biochemical Parameters

The absorbance of all the biochemical parameters was measured in a UV-VIS Spectrophotometer (Shimadzu). After scarification of mice, blood samples were collected, and their liver was excised. The isolated liver rinsed with ice cold normal saline solution followed by cold phosphate buffer having pH 7.4 and blotted dry and weighed. A 10 % w/v homogenate of liver was prepared in ice cold phosphate buffer (pH 7.4) and a portion was utilized for estimation of lipid peroxidation and other portion of the same after precipitation of proteins with TCA was used for estimation of glutathione remaining homogenate were centrifuged at 1500 rpm at 4°C for 15 min. The supernatant thus obtained was used for the estimation of catalase and protein content.

2.10.9. ***Estimation of Lipid Peroxidation Assay***

Malondialdehyde (MDA), a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reactive. Taken 0.2 ml of tissue homogenate; 0.2 ml of 8.1% sodium lauryl sulfate (SLS), 1.5 ml of 20 % acetic acid and 1.5 ml of 0.8 % thiobarbituric acid (TBA) were added in succession. The volume of mixture was made upto 4 ml with distilled water. The mixture was incubated for 60 min at 95°C in a temperature control water bath and cooled and added 5 ml of n butanol:pyridine (15:1) mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min., the upper organic layer was separated, and absorbance was read at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as n moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of 1.56×10^5 cm^{-1}. [30]

2.10.10. Estimation of Catalase Activity

The catalase activity was determined in liver homogenate. After centrifugation of liver homogenate, supernatant 0.05 ml was added to a test tube containing 2 ml of phosphate buffer (pH - 7.4) and 1 ml of 30 mm hydrogen peroxide and mixed well. Catalase activity was measured at 240 nm for 1 min in the time internal of 10 sec against blank using spectrophotometer. The molar extinction coefficient of hydrogen peroxide 43.6 M cm^{-1} was used to determine the catalase activity. One unit of activity is equal to one millimoles of hydrogen peroxide degraded per minute and is expressed as unit per milligram of tissues[31].

2.10.11. Estimation of Total Protein Content

The total serum protein was estimated by modified biuret method. The prepared 10% w/v liver homogenate in phosphate buffer solution (pH 7.4) was used for the estimation of protein content. The prepared homogenate was centrifuged at 1500 rpm for 15 min. at 4° C. The supernatant thus obtained was used for estimation. Test solution was prepared by using 0.2 ml serum, 5 ml biuret reagent and 3 ml distilled water. Standard solution was prepared by using 3 ml solution of bovine albumin and 5 ml biuret reagent. The absorbance of the sample was read against the blank at 540 nm in UV visible double beam spectrophotometer. The amount of protein was expressed in gm of protein in 100 ml. [32].

2.11. Statistical Analysis

The data obtained from animal experiments were expressed as mean ± SEM (Standard Error of Mean). For statistical analysis data were subjected to analysis of variance (ANOVA) followed by Student's t-test. Values are considered statistically significant.

3. RESULTS

3.1. Preliminary Phytochemical screening

The results of preliminary phytochemical screening of different extracts viz Methanolic extract (MEIS), Chlorofom extract (CHIS) and Ethyl acetate extract (EAIS) of *I. singaporensis* is presented in Table 1.0

We found that tannins, saponin glycosides and anthraquinone glycosides are present in the extracts.

3.2. Determination of total antioxidant capacity

Total antioxidant capacity of the different extracts of *I.singaporensis* was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid (R2). Ethyl acetate extract of *I. singaporensis* was found to possess the highest total antioxidant capacity (Fig.1). The antioxidant capacity of the Ethyl acetate (EAIS) extract was found 64.8 mg/g of ascorbic acid. Total antioxidant capacity of the extracts was found low in the methanol extract (52.4mg/g) and chloroform extract (27mg/g) (Fig.1).

3.3. Determination of total flavonoids content

Flavonoids play an important role in antioxidant system in plants. The antioxidant properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of

enzymes responsible for free radical generation. Depending on their structure, flavonoids are able to scavenge practically all known ROS.

Aluminium chloride colorimetric method was used to determine the total flavanoid contents of the different extracts of *I. singaporensis.* Total flavonoid contents were calculated using the standard curve of quercetin (R2) and was expressed as quercetin equivalents (QE) per gram of the plant extract. Ethyl acetate extract of *I. singaporensis* was found to contain the highest amount of flavonoid (18.79 mg/g), whereas in MEIS and CHIS the flavonoid content found 12.9mg/g and 10.90 mg/g, respectively (Fig.2).

3.4. Time and dose dependent action of extracts of I. singaporensis

We found that, all extracts were not inducing antiproliferative or cytotoxic effect on the cells. Figure 3, shows the antiproliferative profiles of the MEIS, CHIS and EAIS against the HeLa cancer cell line. Only the EAIS was found antiproliferative against the HeLa cells with the IC50 values recorded at 26 ± 0.6 µg/ml (Fig.3).

***3.5. In vivo* anticancer activity**

3.5.1. Evaluation of hematological parameters of ethyl acetate extract of I. singaporensis

EAIS extract of three different concentrations were used. The hemoglobin count and the RBC count were significantly increased in all the three extract. The administration of EAIS 200, 300 and 500 mg/kg in EAC bearing mice reduced total WBC count initially. In cancer chemotherapy the major problem is of mylosuppression and anemia. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic conditions. Treatment with EAIS brought back the hemoglobin content, RBC and WBC cell count to near normal values (Fig.4). This indicates that EAIS possess protective action on the hematopoietic system.

3.5.2. Evaluation of biochemical parameters of ethyl acetate extract of Ixora singaporensis

The lipid peroxidation in liver tissues was increased in EAC control group as compared to normal group. After administration of EAIS at different doses (200mg/kg, 300mg/kg and 500mg/kg) to EAC bearing mice, the level of lipid peroxidation was reduced (0.19, 0.14 and 0.09 units/gm respectively). The catalase level in EAC control group decreased compared with normal group (Fig.5). Treatment with different doses of EAIS increased the catalase levels (5.27, 6.23 and 6.86 units/gm respectively). The protein content also decreased with increased dose of EAIS viz 12.95, 11.48 and 10.74 gm/100ml (Fig.5)

The level of lipid peroxidation, catalase and protein contents was summarized in figure 5. Lipid peroxidation mediated by free radical considered being a primary mechanism of cell membrane destruction and cell damage. Present study indicated that EAIS significantly reduced the elevated levels of lipid peroxidation and thereby it may act as an antitumor agent by virtue of its antioxidant mechanism.

3.5.3. Evaluation of effect of EAIS on body weight of tumor bearing mice

Individual body weights were recorded daily during the experimental period. Mean body weight gains were calculated for each group. The EAIS 200 mg/Kg, 300 mg/kg and 500 mg/kg treated animal group were found gaining the body weight, (Fig 6 A).

3.5.4. Evaluation of mean survival time and percentage increase life span in tumor bearing mice

The effect of EAIS on the survival of tumor bearing mice shown in Table 2. The MST of control group was 22 days, whereas it was 26, 29 and 56 days for 200 mg/kg, 300 mg/kg and 500 mg/kg on treated group respectively. The standard drug 5- FU (10mg/kg) treated groups shows 36 days. The increase in life span of tumor bearing mice treated with EAIS doses 200, 300 and 500 mg/kg and 5- FU (10mg/kg) was found to be 18.6%, 32.56 %, 55.81% and 39.21% respectively as compared to cancer control group.

3.5.5. Evaluation of tumor volume, packed cell volume

The tumor cell volume, packed cell volume and viable cell count of the EAC bearing mice treated with different doses of EAIS extract were reduced compared to EAC control group (Fig 6 B). We found that, tumor cell volume was decreased in EAIS 200mg treated animal groups (2.4ml) as compared to untreated control (4.5 ml). The tumor cell volume was found lower in EAIS 500 mg (1.9 ml) (Figure 6 B). The packed cell volume was found lower in the EAIS treated animal groups (2.2-2.6 ml) than the untreated control (4 ml) (Figure 6 C).

3.5.6. Effect on viable and nonviable cell count of tumor bearing mice parameters

Reduction in viable and increased in nonviable towards the normal in tumor host suggest antitumor effect against EAC cell line in mice (Fig 7). In this study, EAIS increased the nonviable cell count (1.24 ml) which suggested that the crude extract have direct relationship with tumor cells as these tumor cells absorb the anticancer drug by direct absorption and this anticancer agent lysis the cells by direct cytotoxic mechanism. The reliable criteria for judging

the value of anticancer drugs are prolongation of life span, decrease of WBC from blood and decrease of tumor volume.

4. DISCUSSION

This study examined Antitumor & antioxidant potential of ethyl acetate extract of Ixora singaporensis (EAIS) hort against Ehrlich ascites carcinoma tumor. The results show that the EAIS promising antitumor and antioxidant action. Many compounds reported from the ixora sp. including triterpenoids and fatty acids, have been isolated from this plant[32].

The antioxidant capacity of the Ethyl acetate (EAIS) extract was found 64.8 mg/g of ascorbic acid compared to the methanol extract (52.4mg/g) and chloroform extract (27mg/g). this antioxidant action of EAIS was due to the amount of flavonoid (18.79 mg/g). In previous reports, it is stated that the high content of phenolic compounds in the flower extract might explain its higher antioxidant capacity of extract. There is evidence of a good correlation between phenolic contents of the different plant part extracts (R2 = 0.9833) and their DPPH scavenging activity [33-35].

The antiproliferative profiles of the MEIS, CHIS and EAIS against the HeLa cancer cell line. Only the EAIS was found antiproliferative against the HeLa cells with the IC50 values recorded at 26 ± 0.6 µg/ml. In previous studies, the effects of Ursolic acid in retarding invasion and metastasis of lung cancer cells, [36] and in inhibiting the initiation, promotion, and metastasis of other types of cancer [37-38] attracted the attention to the therapeutic potential of triterpene from ixora sp.

Similarly, the cytotoxic and antitumour properties of I. coccinea flowers [39]. The cytotoxic effects against two lung cancer (A549 and H460) and four leukemia (K562, Lucena, HL60, and Jurkat) cell lines showed that the most active compounds were the Ursolic acid and the oxidize derivative, thus indicating the importance of hydrophilic moieties[40.]

Present study indicated that EAIS significantly reduced the elevated levels of lipid peroxidation and thereby it may act as an antitumor agent by virtue of its antioxidant mechanism. The increase in life span of tumor bearing mice treated with EAIS doses 200, 300 and 500 mg/kg and 5- FU (10mg/kg) was found to be 18.6%, 32.56 %, 55.81% and 39.21% respectively as compared to cancer control group. We found that, tumor cell volume was decreased in EAIS 200mg treated animal groups (2.4ml) as compared to untreated control (4.5 ml). The tumor cell volume was found lower in EAIS 500 mg (1.9 ml) (Figure 6 B).

Studies on solid tumours in mice showed that the experimental mice subjected to the administration of flower extract failed to develop tumours. The second group that was administered with the purified flower extract 10 days after transplantation of tumour cells developed tumours initially but further growth of tumours was arrested without any decrease in the tumour volume indicating tumour static property of the extract. The packed cell volume was found lower in the EAIS treated animal groups. it was previously mentioned that that the crude extract affecting with tumor cells due to absorption of the compounds from ixora sp. by direct absorption. The phytochemicals are directly inducing apoptosis in the tumors and reducing the tumor size. The reliable criteria for judging the value of anticancer drugs are prolongation of life span, decrease of WBC from blood and decrease of tumor volume.

5. CONCLUSION

The result of present study shows that ethyl acetate extracts of *I. Singaporensis* plant is a promising source of potential anticancer activity with minimal toxicity. The preliminary phytochemical screening of the extract showed the presence of flavonoids and tannins which may be attributed for its anticancer activity. The extract may be efficient as preventive agent in various diseases and can be considered as a natural herbal source as an anticancer agent. However, detailed studies on isolation and identification of phyto-constituents of *I. Singaporensis* leaf extracts and further evaluation is essential to characterize them as potent anticancer drug.

REFERENCES

1. Saleha A, Tahmina H, Esrath J I, Md Faisal K, Shanta A, Taslima B. Comparative antimicrobial activities of different species of Ixora. J Pharmacog and Phytochem. 2015, 3(6): 103-105.
2. Kharat A R, Nambiar V V, Tarkasband Y S, Pujari R R. A review on phytochemical and pharmacological activity of genus ixora. Int J Res in Pharm and Chem. 2013, 3(3): 23-28.
3. Okhale S E, Ugbabe G E, Oladosu P O, Ibra-him J A. Egharevba H O, et al. Chemical constituents and antimicrobial activity of the leaf essential oil of Ixora coccinea L (Rubiaceae) collected from North Central Nigeria. I J of Bioassays. 2018, 7(5): 5630-5637.
4. Joshi A B, Surlikar P M, Bhobe M. Ixora coccinea Linn: Phytochemical Investigation. Int J Res Pharm Chem. 2013, 3(3): 691-696.
5. Nripendra N B, Deb K S, Sudipta C, Partha P M. Comparative study of in vitro free radical scavenging

activity of different leaf extracts of Ixora coccinea L. Int J Biomed Res. 2011, 2(1): 32-40.
6. Mukesh, C S, Smita S, Preliminary Phytochemical and antimicrobial investigations of the aqueous extract of Ixora coccinea L. and Commelina benghalensis L. on Gram-Positive and GramNegative Microorganisms. Middle East J Sci Res. 2010, 6(5): 436-439.
7. Baliga M S, Kurian P J, Ixora coccinea L. traditional uses, phytochemistry and pharmacology. Chin J Integr Med. 2012, 18(1): 72-79.
8. Rohini S, Shalini N, Narayanaswamy Balakrishnan K P. Application of Natural Products In Cosmetics: A Study of Ixora coccinea Extracts for their Antityrosinase and Antioxidant Activities. Int J Res Cos Sci. 2012, 2(1): 1-7.
9. Sankhadip B, Sushomasri M, Pranabesh C. Comparative study of in vitro and in vivo antioxidant property of different Ixora species. J Adv Pharm Edu & Res. 2011, 2: 90-103.
10. Surana A R, Aher A N, Pal S C, Deore. Evaluation of anthelmintic activity of Ixora coccinea. J Pharm Life Sci. 2011, 2(6): 813-814.
11. Naskar M, Bhattacharya S, Biswas M. Antileishmanial effect of Ixora coccinea leaf extracts on the in vitro growth of Leishmania donovani promastigotes. J Adv Pharm Edu Res. 2013, 3(4): 471-474.
12. Missebukpo A, Metwogo K, Agobon A, Eklu Gadegbeku K, Akilikoku K, et al. Evaluation of anti-asthmatic activity of Ixora coccinea. J Pharma Toxicol. 2011, 6(6): 559-570.
13. Yasmeen M, Prabu B. Evaluation of the Hypoglycaemic and Hypolipidaemic activities of the aqueous extract of the leaves of I. coccinea L. in Diabetic Rats. J Clin Diagno Res. 2011, 5(7): 1381-1384.
14. Firoz N M, Bharatesh R K, Tabassum S S, Nilofar S N. Cardioprotective effect of methanolic extract of Ixora coccinea L. leaves on doxorubicininduced cardiac toxicity in rats. Indian J Pharmacol. 2012, 44(2): 178–183.
15. Arunachalam G, Subramanian N, Pazhani P, Karunanithi M, Ravichandran V. Phytochemical and anti-ulcer investigations of the fresh leaf extract of Ixora coccinea L (Rubiaceae) in albino rat model. Int J Pharm Sc. 2009, 1(1): 26-31.
16. Patibandla N B, Nagaraju B, Vinay I. Evaluation of antiulcer and in-vitro antioxidant activities of Ixora coccinea flowers and polyherbal extract in Wistar Albino Rats. Int J Pharm Pharm Sci. 2014, 6(2): 339-344.
17. Harish K S, Srinath R. Evaluation of Neuroprotective Activity of Ixora coccinea extract on experimentally induced Neurotoxicity in Albino Rats. Synopsis 2013. Rajiv Gandhi University of Health Sciences, Bengaluru, Karnataka. 2013.
18. Shan P M, Nasiya L, Sri G P, Evaluation of Anxiolytic activity of Ixora coccinea L. ethanolic extract in Swiss Albino mice. Clin Exp Pharmacol. 2014, 4(146): 1-3.
19. OECD. OECD guidelines for the testing of chemicals. Tumor cell growth response. 2008, 1-27.
20. Tian S, Shi Y, Zhou X, Ge L, Upur H. Total polyphenolic (flavonoids) content and antioxidant capacity of different Ziziphora clinopodioides Lam. extracts. Pharmacogn Mag. 2011, 7(25): 65–68.
21. Kumar S, Sandhir R, Ojha S. Evaluation of antioxidant activity and total phenol in different varieties of Lantana camara leaves. BMC research notes. 2014, 7: 560.
22. Asma, H S, Amzad M H. Total phenols, total flavonoids contents and free radical scavenging activity of seeds extracts of pigeon pea traditionally used in Oman for the treatment of several chronic diseases. Asian Pac J Trop Dis. 2015, 5(4): 316-321.
23. Roham P H, Kharat K R, Mungde P, Jadhav, M A, Makhija S J. Induction of mitochondria mediated apoptosis in human breast cancer cells (T-47D) by Annona reticulata L. Leaves Methanol. Extr. Nutr. Canc. 2016. 68: 305–311.
24. Kharat A R, Tarkasband Y S, Kharat K. Antitumor Potential of Ethyl Acetate Extract of Kirganelia reticulata Against Ehrlich Ascites Carcinoma Tumor. J of Biol Act Prod from Nat. 2016, 6(4): 337-346.
25. Singh J, Kumar A, Rajapandi R, Ghosh T, Mondal A, Maiti B C, Maity T K. synthesis and anticancer activity of some 1, 3, 4-oxadiazole derivatives against ehlirch ascites carcinoma bearing mice model. Pharmacol. online. 2010, 1: 406-416.
26. Freshney R I, Culture of Animal Cells. a Manual of Basic Technique. 5th Edition, John Wiley & Sons, Hoboken. 2005.
27. D'Armour, F E, Blood FR, Belden, D A. The manual for laboratory work in mammalian physiology. 3rd ed. Chicago: The University of Chicago Press. 1965, : p. 4-6.
28. Wintrobe M M, Lee G R, Boggs D R, Bithel T C, Athens J W, Foerester. Clinical Hematology 5th ed. Philadelphia. 1961, p. 326.
29. Dacie, J V, Lewis S M. Practical hematology. 2nd ed. London: J and A Churchill. 1958, : p. 38-48.
30. Ohkawa H, Ohishi N, Yaki K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979, 95: 351–8.
31. Maehly A C, Chance B. In: Methods of Biochemical Analysis. Vol. I, Glick D editor. New York: Interscience. 1954: p. 357.
32. Lowry O H, Rosebrough N J, Farr A L, Randall R J. Protein measurement with the Folin-phenol re. 1951, 193: 265-75.
33. Versiani M, Ikram A, Khalid S, Faizi S, Tahiri I. Ixoroid: A New Triterpenoid from the Flowers of Ixora coccinea. Nat. prod. comm. 2012, 7: 831-4.
34. Angeline T, Sreenivasan S, Lachimanan YL, Sivaramakrishnan S, Surash R. Antioxidant activity and total phenolic content of methanol extracts of Ixora coccinea. Pharma Biol. 2010, 48(10): 1119-1123.
35. González E M, de Ancos B, Cano M P. Relation between bioactive compounds and free radical-scavenging capacity in berry fruits during frozen storage. J. Sci. Food. Agr. 2003, 83: 722–726.
36. Makris D P, Boskou G, Andrikopoulos N K, Polyphenolic content and in vitro antioxidant characteristics of wine

industry and other agri-food solid waste extracts. J Food Comp Anal. 2007, 20: 125–132.

37. Alves M S, Maciel M, Vega R G, de Mello H, de Araújo Martins C, Esteves-Souza A, Gattass C R, Echevarria A. Ultrasound-assisted Extraction of Ursolic Acid from the Flowers of Ixora coccinia Linn (Rubiaceae) and Antiproliferative Activity of Ursolic Acid and Synthesized Derivatives. Pharmacog. mag. 2017, 13(50): 265-269.
38. Huang C Y, Lin C Y, Tsai C W, Mei C. Inhibition of cell proliferation, invasion and migration by ursolic acid in human lung cancer cell lines. Toxicol. In vitro. 2011, 25: 1274–80.
39. Shanmugam M K, Dai X, Kumar A P, Tan B K H, Sethi G, Bishayee A. Ursolic acid in cancer prevention and treatment: Molecular targets, pharmacokinetics and clinical studies. Biochem Pharmacol. 2013, 85: 1579–87.
40. Latha P G, Panikkar K R, Cytotoxic and antitumor principles from Ixora coccinea flowers. Cancer Lett. 1998, 130: 197–202.