Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2014; 2(12A): 854-859 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI:** 10.36347/sajb.2014.v02i12.002

Research Article

Phytochemical Screening and In vitro Evaluation of Cytotoxic Activity of Fruits of *Lagerstroemia speciosa L.*

Asish Bhaumik[1*]; M. Upender Kumar[1]; Ch. Monogna[1]; Kaleem Ahmed Khan[2]; M. Uttara[2]; M. Swapna[2]
[1]Department of Pharmaceutical Chemistry, [2]Department of Pharmacology, Teja College of Pharmacy, Kodad, Nalgonda-508206, Telangana State, India

***Corresponding author**
Asish Bhaumik
Email: bhaumik.asish@gmail.com

Abstract: The objective of the present work is to evaluate the cytotoxic activity of fruits (without seeds) of *Lagerstroemia speciosa L.* Based on this, a new series of constituents had been planned to extract by Methanol (ML), Ethanol (EL), Chloroform (CF) from the fruits of *Lagerstroemia speciosa L*. The *in-vitro* cytotoxic activities were carried out against two human cancer cell lines such as Human Acute Monocytic Leukemia- HL- 60 cell line and Human Colon Cancer- DLD-1 cell line and MTT assay was used to analyze the cell growth inhibition of the both and doxorubicin was used as a standard drug for the HL-60 cell line and 5- Flurouracil was used for DLD-1 cell line. The results showed that the various extracts of fruits of *Lagerstroemia speciosa L* had a very good to moderate cytotoxic activity against both HL- 60 and DLD-1 cell lines and it was also reported that the CF extracts had shown the highest cytotoxic activity against both cell lines among the three extracts. The IC_{50} values of the various extracts of fruits of *Lagerstroemia speciosa L* were found to be ML (IC_{50} of 3.4µg/ml), EL (IC_{50} of 3.9µg/ml), CF (IC_{50} of 2.3µg/ml) for HL-60 cell line and ML (IC_{50} of 3.2µg/ml), EL (IC_{50} of 3.8µg/ml), CF (IC_{50} of 2.2µg/ml) for DLD-1 cell line.
Keywords: *Lagerstroemia speciosa L*, Cytotoxic activity, HL-60, DLD-1, MTT assay, IC50

INTRODUCTION

Jarul (it is also known as Banaba) is a flowering plant that grows in warm climate like the Philippines, India and others. Jarul is widely used in the Philippines and as herbal medicine for diabetes. While in India, Jarul is also used in Ayurvedic medicine for the treatment of diabetes. The Jarul leaves and flowers contain corrosolic acid, a substance being studied for its insulin like effect of lowering the glucose in the body. Jarul is also being studied as a weight-loss supplement for its ability to delay or reduce the absorption of carbohydraes. Jarul is also rich in vitamins and minerals including zinc and magnesium. Jarul is also rich in dietary fibers. The scientific name the Jarul or Banaba is *Lagerstroemia speciosa L(*Lythraceae). It displayed that the leaves, bark, stem seeds of *Lagerstroemia speciosa L* was found to be contain several bioactive compounds such as terpenoids, steroids, saponins, flavonoids and glycosides and alkaloids etc[1].

The genus Lagerstroemia was first described by Carlos Linneaus. The name Lagerstroemia recognizes Magnus von Lagerstroem, a Swedish naturalist who provided specimens from the East for Linnaeus. It is a small to medium-sized tree growing to 20 metres (66 ft) tall, with smooth, flaky bark. The leaves are deciduous, oval to elliptic, 8–15 cm (3.1–5.9 in) long and 3–7 cm (1.2–2.8 in) broad, with an acute apex. The flowers are produced in erect panicles 20–40 cm (7.9–15.7 in) long, each flower with six white to purple petals 2–3.5 cm (0.79–1.38 in) long. Folkloric uses of Banaba herbal medicine include the treatment for diarrhea, constipation, inflammation of kidneys, dysuria and other urinary dysfunctions. Banaba is a tropical flowering tree that grow up to 10 meters high. Banaba has large green oblong leaves that is about 3 inches in width and 7 inches in length. The flowers or Banaba are racemes and colored pink to lavender. Banaba bears nut-like fruits that are arranged in large clumps.It is grown in South East Asia, India and the Philippines. It is also widely cultivated as an ornamental plant in tropical and subtropical areas [2].

Banabá has a long history of folkloric medical applications that include blood pressure control, urinary dysfunctions (helps ease urination), cholesterol level control, treatment of diarrhea, facilitates bowel movement, diabetes and as an analgesic [3]. The chemical compounds that have been isolated from the extract include corosolic acid, lager-stroemin, flosin B, and reginin A. The leaves of the Banabá and other parts are used widely in the Philippines, Taiwan, and Japan as a tea preparation. Banabá herb is one of the 69 herbal

plants promoted by the Philippine Department of Health (DOH) [3].

Lagerstroemia speciosa have been previously reported to have hypoglycemic activity by reducing fasting blood glucose of strptozocin induced Diabetic rats. Apart from hypoglycemic activity **[**4-5]. Banava leaves also possessed Antioxydant [6], Anti inflammatory [7], Antiobesity [8] and Antifibrotic [9].

The cytotoxic activity of fruits of *Lagerstroemia speciosa* was not investigated till now, so the main objective of the present research work is to screen the phytoconstituents and evaluate the *in vitro* cytotoxic activity of fruits (exo and endo carp) of *Lagerstroemia speciosa L.*

MATERIALS AND METHOD:

Chemicals and drugs:

The all chemicals used for the extraction and phytochemical screening were of LR and AR grade.

Cell culture :

The Human Acute Monocytic Leukemia- HL- 60 cell line and Human Colon Cancer DLD-1 cell line were provided by National Centre for Cell Science (NCCS), Pune and were grown in Eagles Minimum Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 100% relative humidity, 5% CO2, 95% air and the culture medium was changed twice a week. The standard drug doxorubicin and 5-FU were purchased from Local Whole sale Pharmacy shop and other chemicals and solvents were used from Institutional store and were of AR grade.

Apparatus and chemicals required:

Round bottom flask, water condenser, heating mantle, motor and pestle, methanol, ethanol, chloroform, dichloromethane, sodium chloride solution, magnesium sulfate etc.

Extraction

Weigh 50 g of fruits of *Lagerstroemia speciosa* (unripe and ripen can be mashed to prepare a paste) into a 500 ml round-bottomed flask. Add 200 ml of methanol and 240 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing bioactive compounds with three portions of 250 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating. The same procedure have been followed for the preparation of EL and CF extracts.

Preliminary Phytochemical screening [10-11] :

Preliminary phytochemical screening of various extracts **(**ML, EL and CF) of fruits of *Lagerstroemia speciosa* had shown the presence of following bioactive compounds which were confirmed by their specific qualitative confirmatory chemical tests: Proteins and amino acids, Carbohydrates, Glycosides, Alkaloids, Terpenoids, Saponins, Phytosterols, Flavanoids, Gum and mucilage etc.

In-vitro evaluation of cytotoxic activity by MTT assay [12-13]

Cell culture

The Human Acute Monocytic Leukemia- HL- 60 cell line and Human Colon Cancer DLD-1 cell line were provided by National Centre for Cell Science (NCCS), Pune and was grown in Eagles Minimum Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained at 37°C, 100% relative humidity, 5% CO2, 95% air and the culture medium was changed twice a week.

Cell treatment

The monolayer cells were detached and single cell suspensions were made using trypsin-ethylenediamine tetraacetic acid (EDTA). A hemocytometer was used to count the viable cells and the cell suspension was diluted with a medium containing 5% FBS in order to obtain final density of $1x10^5$ cells/ml. 96-well plates at plating density of 10,000 cells/well were seeded with one hundred microlitres per well of cell suspension and incubated for cell attachment at 37° C, 5% CO2, 95% air and 100% relative humidity. The cells were treated with serial concentrations of the test samples after 24 hr. Serial dilution method was used for preparing test samples of different concentrations. Cells were initially dissolved in dimethylsulfoxide (DMSO) and further diluted with serum free medium to obtain twice the desired final maximum test concentration. The required final extract concentrations of 200, 100, 50, 25, 12.5 and 6.25 µg/ml were obtained by adding aliquots of 100 µl of the different drug dilutions to the appropriate wells already containing 100 µl of medium. After addition of the extract the plates were incubated for an additional 48 hr at 37° C, 5% CO2, 95% air and 100% relative humidity. The medium without samples served as control and triplicate was maintained for all concentrations.

MTT assay

After 48h of incubation, to each well 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added and incubated at 37° C for 4h. The medium with MTT was flicked off and the formed formazan crystals were solubilized in 100µl of DMSO. Using micro plate reader the absorbance was measured at 570 nm. The % cell inhibition was determined using the following formula. % Cell Inhibition = [100- Abs (sample)/Abs (control)] x100.

RESULT AND DISCUSSION:

The results for cell growth inhibition by the various extracts (ML, EL, CF) of fruits of *Lagerstroemia*

speciosa against both Human Acute Monocytic Leukemia- HL- 60 cell line and Human Colon Cancer - DLD-1 cell line for various concentrations were shown in table 1, 2, 3, 4, 5, 6, 7 and 8. As the concentration increases there was an increased in the cell growth inhibition and it was found that the extract CF possessed the highest cytotoxic activity against both cell lines. The IC_{50} values of the various extracts of fruits of *Lagerstroemia speciosa L* were found to be ML (IC_{50} of 3.4µg/ml), EL (IC_{50} of 3.9µg/ml), CF (IC_{50} of 2.3µg/ml) for HL- 60 cell line and ML (IC_{50} of 3.2µg/ml), EL (IC_{50} of 3.8µg/ml), CF (IC_{50} of 2.2µg/ml) for DLD-1 cell line were more than 100µg/ml and the regression values were difficult to analyze.

Table 1 : For percentage (%) of cell Growth Inhibition of Methanolic Extract (ML) of Fruits of *Lagerstroemia speciosa* on HL-60 Cell lines by MTT Assay:

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	6.25 µg/ml	1.419	88.1
2	12.5 µg/ml	1.460	83.2
3	25 µg/ml	1.52	78.2
4	50 µg/ml	1.54	70.1
5	100 µg/ml	1.568	67.1
6	200 µg/ml	1.612	59.1
7	Control	1.801	0

Table 2 : For percentage (%) of cell Growth Inhibition of Ethanolic Extract (EL) of Fruits of *Lagerstroemia speciosa* on HL-60 Cell lines by MTT Assay:

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	6.25 µg/ml	1.422	82.2
2	12.5 µg/ml	1.470	75.5
3	25 µg/ml	1.61	66.9
4	50 µg/ml	1.63	65.4
5	100 µg/ml	1.612	59
6	200 µg/ml	1.720	39.9
7	Control	1.801	0

Table 3 : For percentage (%) of cell Growth Inhibition of Chloroform Extract (CF) of Fruits of *Lagerstroemia speciosa* on HL-60 Cell lines by MTT Assay:

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	6.25 µg/ml	1.412	93.1
2	12.5 µg/ml	1.450	87.1
3	25 µg/ml	1.51	79.2
4	50 µg/ml	1.53	73.1
5	100 µg/ml	1.551	69.1
6	200 µg/ml	1.599	62.8
7	Control	1.801	0

Table 4 : For percentage (%) of cell Growth Inhibition of Standard drug Doxorubicin on HL-60 Cell lines by MTT Assay:

Serial no.	Concentration of the Doxorubicin	Absorbance of Doxorubicin	Inhibition of cell growth (%)
1	6.25 µg/ml	1.101	97.2
2	12.5 µg/ml	1.21	90.4
3	25 µg/ml	1.301	89.1
4	50 µg/ml	1.309	85.5
5	100 µg/ml	1.399	70.1
6	200 µg/ml	1.4	68.9
7	Control	1.704	0

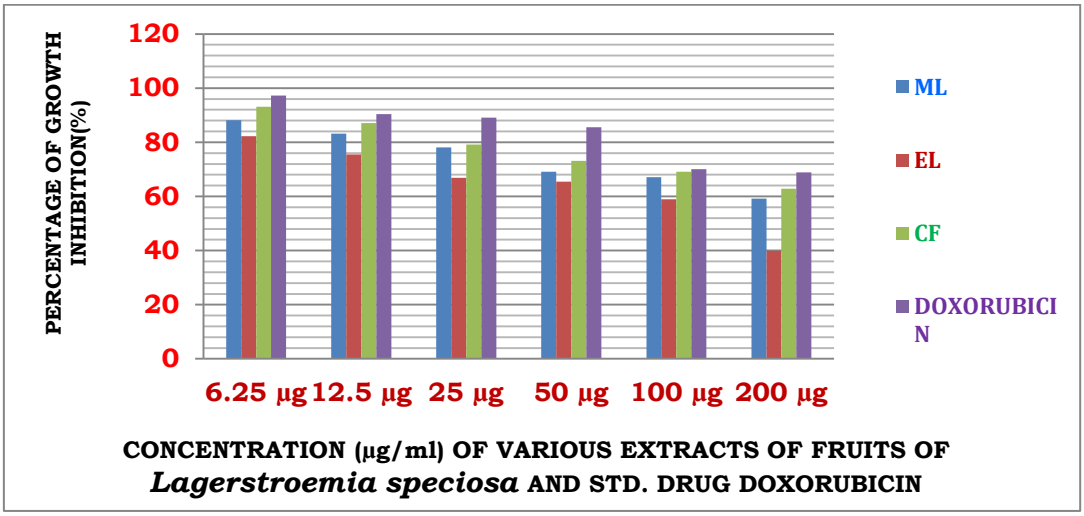

FIG 1: Cytotoxic activity of various extracts of Fruits of *Lagerstroemia speciosa* against HL-60 Cell lines Concentration Vs % growth inhibition.

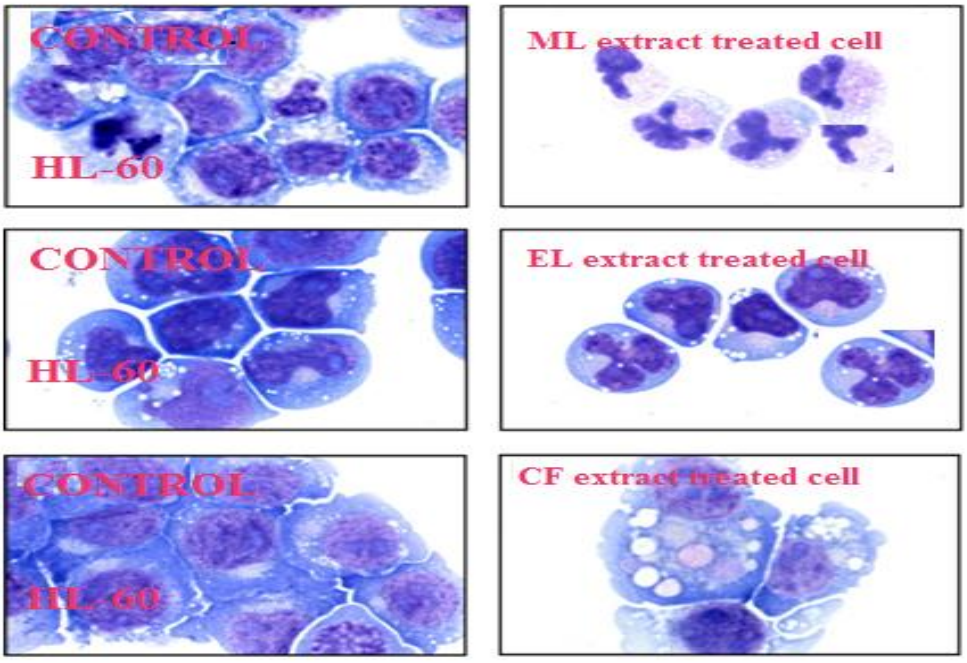

FIG 2 : Cytotoxic activity of various extracts of Fruits of *Lagerstroemia speciosa* against HL-60 Cell lines for % growth inhibition.

Table 5 : For percentage (%) of cell Growth Inhibition of Methanolic Extract (ML) of Fruits of *Lagerstroemia speciosa* on DLD-1 Cell lines by MTT Assay:

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	6.25 µg/ml	1.417	90.1
2	12.5 µg/ml	1.460	80.9
3	25 µg/ml	1.49	70.9
4	50 µg/ml	1.58	62.9
5	100 µg/ml	1.61	57.9
6	200 µg/ml	1.71	45.5
7	Control	1.709	0

Table 6 : For percentage (%) of cell Growth Inhibition of Ethanolic Extract (EL) of Fruits of *Lagerstroemia speciosa* on DLD-1 Cell lines by MTT Assay:

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	6.25 µg/ml	1.422	87.8
2	12.5 µg/ml	1.468	79.9
3	25 µg/ml	1.56	68.8
4	50 µg/ml	1.60	59.7
5	100 µg/ml	1.69	55.5
6	200 µg/ml	1.79	41.1
7	Control	1.709	0

Table 7 : For percentage (%) of cell Growth Inhibition of Chloroform Extract (CF) of Fruits of *Lagerstroemia speciosa* on DLD-1 Cell lines by MTT Assay:

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	6.25 µg/ml	1.416	94.1
2	12.5 µg/ml	1.409	89.9
3	25 µg/ml	1.54	75.1
4	50 µg/ml	1.59	66.1
5	100 µg/ml	1.61	57.1
6	200 µg/ml	1.610	49.9
7	Control	1.709	0

Table 8 : For percentage (%) of cell Growth Inhibition of Standard drug 5-FU on DLD-1 Cell lines by MTT Assay:

Serial no.	Concentration of the 5-FU	Absorbance of 5-FU	Inhibition of cell growth (%)
1	6.25 µg/ml	1.201	96.9
2	12.5 µg/ml	1.302	90.4
3	25 µg/ml	1.399	89.8
4	50 µg/ml	1.4	84.4
5	100 µg/ml	1.41	76.6
6	200 µg/ml	1.420	68.5
7	Control	1.601	0

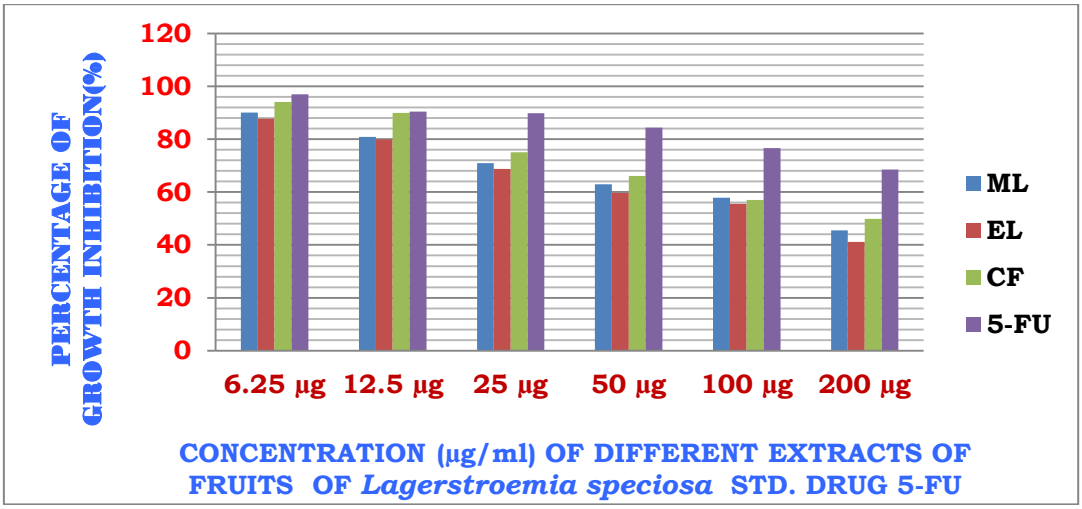

FIG 3 : Cytotoxic activity of different extracts of Fruits of *Lagerstroemia speciosa* against DLD-1 Cell lines Concentration Vs % growth inhibition.

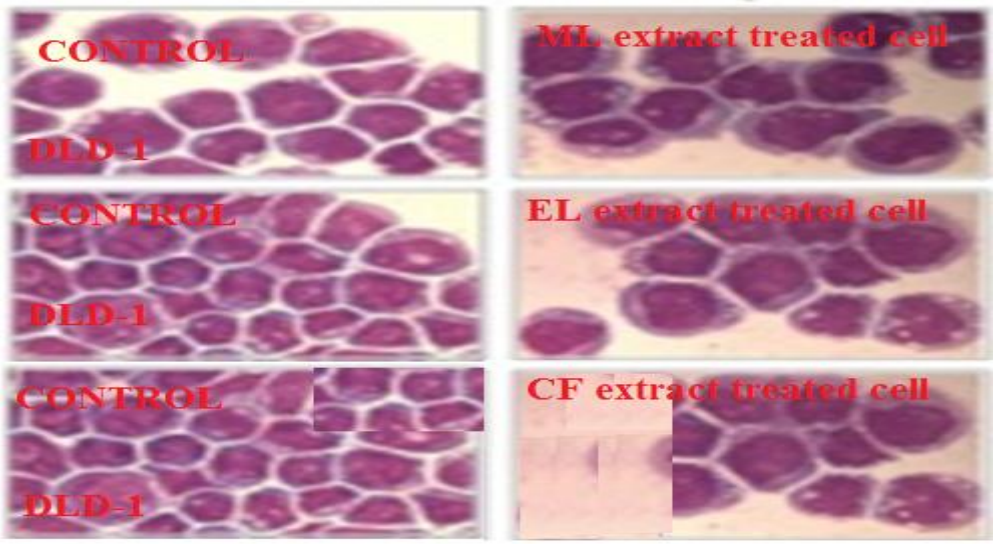

FIG 4 : Cytotoxic activity of various extracts of Fruits of *Lagerstroemia speciosa* against DLD-1 Cell lines for % growth inhibition.

CONCLUSION

In conclusion, we report here that the various extracts of fruits of *Lagerstroemia speciosa L* had the ability to kill tumour cells in vitro. The cytotoxic activities of the extracts ML, EL and CF against both HL- 60 and DLD-1 cell lines can be considered very good with regards to the USNCI standard. It was also displayed that among these three extracts CF extract was found to be possess the highest cytotoxic activity with growth of inhibition 93.1% for HL-60 and 94.1% for DLD-1 cell lines at the highest concentration 6.25 µg/ml, then ML extract with growth of inhibition 88.1%, 90% for HL-60 and DLD-1 and lastly EL extract with growth of inhibition 82.2%, 87.8% for HL-60 and DLD-1 cell lines at the highest concentration 6.25 µg/ml.

REFERENCES

1. Banaba Uses, Health Benefits, Side Effects. Available from http://www.medicalhealthguide.com/articles/banaba.htm
2. Lagerstroemia duperreana. Available from http://ntbg.org/plants/plant_details.php?plantid=6832
3. Lagerstroemia speciosa. Available from http://en.wikipedia.org/wiki/Lagerstroemia_speciosa
4. Saha BK, Bhuiyan MNH, Mazumder K, Haque KF; Hypoglycemic activity of Lagerstroemia speciosa L. extract on streptozotocin-induced diabetic rat: Underlying mechanism of action. Bangladesh Journal of Pharmacology, 2009; 4(2):79-83.
5. Deocaris CC, Aguinaldo RR, dela Ysla JL, Asencion AS, Mojica ERE; Hypoglycemic activity of irradiated banaba (Lagerstroemia speciosa Linn.) leaves. Journal of Applied Sciencse Research, 2005; 1(1): 95-98.
6. Unno T, Sakane I, Masumizu T, Kohno M, Kakuda T; Antioxidative activity of water extracts of Lagerstroemia speciosa leaves. Bioscience, biotechnology, and biochemistry, 1997; 61(10):1772-1774.
7. Priya TT, Sabu Mc, Jolly C; Free radical scavenging and Anti inflammatory properties of Lagerstroemia speciosa. Inflammatory Pharmacology, 2008; 16 : 128-187
8. Klein G, Kim J, Himmeldirk K, Cao Y, Chen X; Antidiabetes and anti-obesity activity of Lagerstroemia speciosa. Evidence-Based Complementary and Alternative Medicine, 2007; 4(4): 401-407.
9. Vinoth P; Evaluation of Anti fibrotic effect of Lagerstroemia speciosa L pers. On carbon tetra chloride Induced liver fibrosis. Current Pharma Research, 2010 ; 1 : 56-68.
10. Satyanarayana U, Chakrapani U; Biochemistry, 3rd edition ,PP- 9-10, 43-44.
11. Kokate CK, Purohit AP, Gokhale SB; Pharmacognosy, 42 Edition, pp- A.1
12. Patel S, Gheewala N, Suthar A, Shah A; In-vitro cytotoxicity activity of Solanum nigrum extract against Hela cell line and Vero cell line. International journal of pharmacy and pharmaceutical sciences, 2009; 1(1):38-46.
13. Shokrzadeh M, Azadbakht M, Ahangar N, Hashemi A; Cytotoxicity of hydro-alcoholic extracts of Cucurbita pepo and Solanum nigrum on HepG2 and CT26 cancer cell lines. Pharmacognosy magazine, 2010; 6(23): 176.