

Essential Oils Composition and Cytotoxic Effect of *Lagerstroemia speciosa* Linn flowers

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ABSTRACT

The chemical compositions of essential oils from *Lagerstroemia speciosa* L flowers obtained by hydrodistillation yielded 0.085% and was analysed by GC and GC-MS. Out of forty five compounds identified the major volatiles in flowers were α -pinene, myrcene, α -terpineol, a β -bisabolene etc. Our findings demonstrate for the first time the potential benefits of this medicinal plant flower as a rich source of high therapeutic value compounds for medicines, cosmetics, supplements and as a health food. As concerning volatile fraction, that can be often a helpful tool to discriminate between different taxa also. Cytotoxicity assay was carried out using Dalton's Lymphoma Ascites cells (DLA) and Ehrlich Ascites Carcinoma cells (EAC). *L. speciosa* essential oil at a concentration of 50 μ L/mL produced 13.33% and 31% cytotoxicity to DLA and EAC cells respectively.

INTRODUCTION

Lagerstroemia speciosa L. (*L. speciosa*) belonging to the family Lythraceae, commonly called as banaba (queen's flower or Crapemyrtle), the Pride of India is a medicinal and ornamental handsome deciduous small tree native of East Asia, India, China and Australia commonly cultivated in gardens throughout for beautiful flowers. Crape myrtle blooms from May through August in India.

Banaba tea is a traditional health drink in Philippines since ancient times. It was found to have therapeutic effects on various ailments such as diabetes, kidney and other urinary problems. Besides being an exotic health drink, banaba tea has a soothing and pleasant taste and Japanese nowadays consume banaba in various forms such as tea, juice, health drinks, and as food supplement. The potential antidiabetic activity of ethyl acetate and ethanolic extracts were studied in the leaves of *L. speciosa* [1,2] Nephroprotective [3], free radical scavenging, anti-inflammatory [4] and hepatoprotective activity [5] of *Lagerstroemia speciosa* (L) extracts were studied recently.

L. speciosa seeds contain caprylic, lauric, myristic, palmitic, steric, arachidic, behenic, lignoceric, oleic, and linoleic acids in the oil [6]. The active principles 9-keotocadec-cis-11-enoic acid has been isolated from seed oil as well [7]. Chemical investigation of amino acid components in banaba seed oil has been performed in earlier studies [8]. Components nonanedioic acid, 12-acetyloxy-9-octadecenoic acid, and 16-methyl-heptadecandic acids present in seed extracts have been identified as having antibacterial activity [9].

An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. An oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant. The natural products industries are currently looking for natural therapeutics and preservatives that can replace synthetic preparations. The scientific literature has identified new applications and uses of both traditional and exotic essential oils. Interest in essential oils has revived in recent decades with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by essential oils have curative effects.

There is a tremendous historical legacy in folklore use of plant preparations in medicine. Novel, safer and effective compounds with cytotoxic and anti tumour activities will be of immense clinical benefit. A wide variety of biologically active compounds from plants have been used as effective chemotherapeutic agents [10]. Around 60% of the current anticancer drugs, in use today are of natural origin. Vinblastine, vincristine, etoposide, teniposide, taxol, topotecan and irinotecan are some of the approved anticancer drugs.

The diverse therapeutic potential of essential oils has drawn the attention of researchers to test them for anticancer activity. From leaves of *L. speciosa*, a new triterpenoid was isolated along with four known compounds of virgatic acid, corosolic acid, ursolic acid and β -sitosterol glucoside ^[11] Using the brine shrimp (*Artemia salina*) lethality bioassay, the ethanol fruit extract of *L. speciosa* showed prominent cytotoxic activity ^[12].

The present study was aimed to identify the components from the essential oils of the *L. speciosa* flowers and to screen the cytotoxicity for which has not explored yet to our knowledge.

MATERIALS AND METHODS

Plant Materials

The flowers of *Lagerstroemia speciosa* L. were collected from the premises of Amala Ayurvedic Hospital, Thrissur, Kerala, India. The plant material was identified by Dr. C.N. Sunil, Department of Botany, of S.N.M. College, Maliankara and was authenticated at Botanical Survey of India, Coimbatore. The voucher specimen (*L. speciosa* BSI No. 62373) has been kept in Fr. Gabriel Herbarium, Amala Ayurvedic Hospital and Research Centre, Thrissur, Kerala, India.

Cell Lines

Dalton's Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) cells were inoculated in peritoneal cavity of Swiss albino mice at Amala Cancer Research Centre. Weekly intraperitoneal inoculation of 10^6 cells in Swiss albino mice was done to maintain the cell-line. Animal experiment was carried out after approval by Institutional Animal Ethics Committee (Reg. No. 149/1999/CPCSEA) and followed the guidelines of CPCSEA, Govt. of India.

Isolation of Essential Oil

Essential oils were obtained by hydrodistillation of fresh flowers of *L. speciosa* using a Clevenger-type apparatus for 6 h and n-Hexane (10 mL) was used as the collector solvent. After evaporation of the solvent, the oil was dried over anhydrous sodium sulphate and stored in sealed vials protected from the light at -20°C before analyses.

Identification and Quantification of Volatile Components

The identification of volatile components was based on computer matching with the WILEY275 and NIST05 libraries, as well as by comparison of the mass spectra and retention indices (RI) with those reported in the literature. In addition, a home-made library, constructed based on the analyses of reference oils and commercial available standards, was used as well.

Gas Chromatography (GC) Analysis

Essential oil (10 μl) was dissolved in hexane (100 μl) and 2 μl of the solution was injected into a GC-17A with Flame Ionization Detector (FID) and C-R6A-chromatopac integrator (Shimadzu Co, Japan). The column used was SUPELCOWAX fused silica (film thickness: 0.2 μm , Supelco USA, 60 m x 0.25 mm). Column temperature was programmed to range from 40 to 250 $^\circ\text{C}$ with a rate of 6 $^\circ\text{C}/\text{min}$. Injector and detector temperatures were 220 and 280 $^\circ\text{C}$, respectively. The carrier gas was hydrogen. Quantification was carried out by % peak area calculations (GC/FID using a non-polar Column).

Gas Chromatography – Mass Spectrometry

For GC/MS analysis, a GC-17A with a QP5050 mass spectrometer (Shimadzu Co, Japan) was used. Helium was used as carrier gas at a flow rate of 0.6 ml/min; the mass spectrometer was operated at 70 eV. Column temperature was programmed from 70 to 200 $^\circ\text{C}$ at 10 $^\circ\text{C}/\text{min}$; injector temperature was 250 $^\circ\text{C}$. Mass spectra correlations were carried out with Wiley, NBS, NIST and private aroma library spectra. Identifications were determined by comparing the retention indices Kovats Index (KI), of each compound with that of known compounds ^[13].

Cytotoxicity assay- Trypan blue exclusion method

The dye exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue whereas dead cells do not. Tumor cells were aspirated from peritoneal cavity and pelleted by centrifugation (1000 $\times g$, for 5 min). The cells were washed with sterile phosphate-buffered saline (PBS) and counted and made up at a concentration of 10 million cells per millilitre. DLA cells (1 million cells/0.1 mL) were incubated with essential oils (dissolved in DMSO) at different concentrations in a total volume of 1 mL made up with PBS. Cells were incubated at 37 $^\circ\text{C}$ for 3 h. After incubation, 0.1 mL of trypan blue (1%) was added and cytotoxicity was determined by counting live and dead cells using a haemocytometer ^[14].

$$\% \text{Cell Death} = \frac{\text{No. of dead cells}}{\text{No. of viable cells} + \text{No. of dead cells}} \times 100$$

RESULT

The chemical composition of the oil of *L. speciosa* flowers was investigated using both GC and GC-MS techniques. Hydrodistillation of the sample yielded pale yellow oil (0.085%).

The components identified from the essential oil and their retention indices (RI), and the percentages are shown in Table 1. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using the NBS54K.L and WILEY.L built in libraries and with those reported in the literature. Comparison of the fragmentation patterns in the resulting mass spectra with those published in literature and using mass spectral database of the gas chromatograph computer is represented as (a,b) in Table 1.

Table 1. Chemical constituents of the Essential oil from the flowers of *L. speciosa*.

Identified Compounds	GC components % area <i>L. speciosa</i>	K _i	
α-pinene	10.38	932	a,b
β-pinene	8.45	976	a,b
myrcene	6.76	990	a,b
limonene	2.60	1027	a,b
<i>Cis</i> - β-ocimene	1.33	1039	a
<i>trans</i> - β ocimene	2.12	1048	a
linalool	1.22	1095	a,b
terpinolene	0.16	1099	a
8-hydroxy linalool	1.12	1186	a,b
α-terpineol	12.76	1187	a
Benzene acetaldehyde	tr	1041	a
unknown	0.47	1086	a
borneol	2.18	1166	a,b
p-cymen-8-ol	0.70	1180	a,b
<i>cis</i> -dihydrocarvone	1.23	1192	a
<i>trans</i> -dihydrocarvone	1.7	1199	a
α -copaene	1.14	1370	a
γ-Elemene	0.38	1430	a,b
unknown	1.96	1456	a
humulene	0.89	1472	a,b
<i>a</i> β-bisabolene	5.97	1508	a
γ-cadinene	tr	1513	a
β-selinene	3.54	1519	a,b
δ-cadinene	2.47	1524	a
caryophyllene oxide	1.69	1581	a,b
Humulene oxide	0.79	1595	a,b
α -bisabolol	3.14	1681	a,b
Unknown	0.97	1695	a
Nootkatone	1.90	1742	a,b

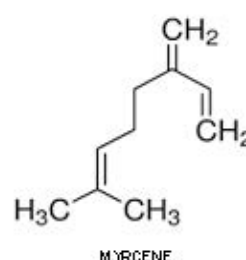
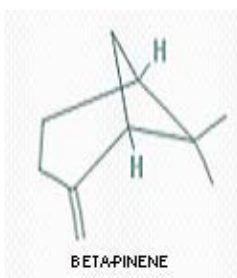
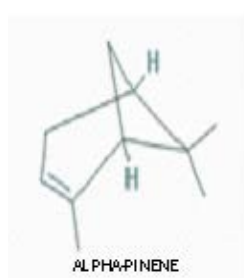
a = Comparison of mass spectra with MS- libraries and retention times

b = Comparison with authentic compounds

tr = concentration less than 0.05%

c = KI, Kovat's indices calculated against C8 to C24 *n*-alkanes

The results showed that *L. speciosa* flower oil contains the monoterpenes like α-pinene, β-pinene, myrcene etc. α-pinene (10.38%), β-pinene, (8.45%) myrcene (6.76%), limonene (2.6%), α -bisabolol (3.14%) etc as major components. *L. speciosa* oil contains (*a* β) bisabolene of 5.97 % and 1.9% percentage of nootkatone. Also peaks of some unknown compounds were identified in this oil.



The cytotoxicity assay was carried out using trypan blue exclusion method and the percentage of dead cells encountered with essential oil treatment at different volumes of oil is shown in Figure 1. *L. speciosa* essential oil at a concentration of 50 $\mu\text{L}/\text{mL}$ produced 13.33% and 31% cytotoxicity to DLA and EAC cells respectively. This result emphasized that the oil possesses cytotoxicity at higher concentration. The results obtained from the present study show that the essential oil from *L. speciosa* is cytotoxic to DLA and EAC cell lines. Our results revealed 77.02% of the constituents and more detailed analysis will help to find out the unknown compounds which may be more active. Of the 45 compounds identified, those present in the largest quantities were α -pinene, myrcene, α -terpineol, a β -bisabolene etc.

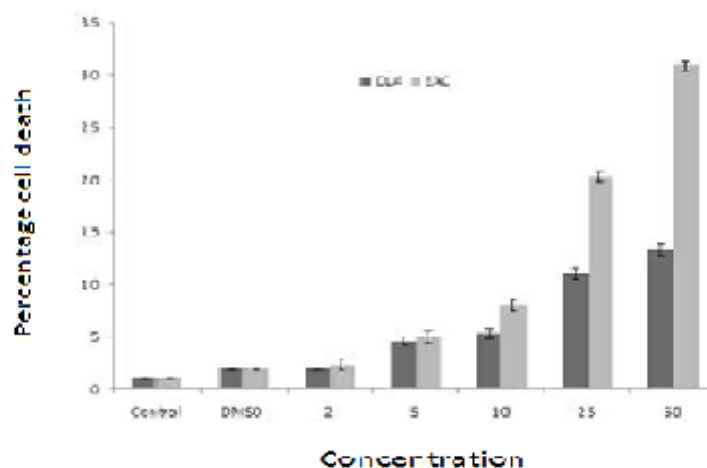


Figure 1. Cytotoxic effects of the oils of *L. speciosa* flower against DLA and EAC cells.

DISCUSSION

This study describes the chemical composition of essential oil obtained from the flowers of *Lagerstroemia speciosa*. Studies have shown differential sensitivities to several natural compounds between tumor and normal cells in vitro or in vivo, and the results obtained from the present study shown that the essential oil from *L. speciosa* is cytotoxic to DLA and EAC cell lines.

Lymphoma is a disease of the lymphocytes in the lymphatic system, which includes the spleen, thymus, as well as other lymphatic tissues. Both EAC and DLA are rapidly growing transplantable tumor cells with aggressive behavior [15]. Dalton's ascites lymphoma is transplantable, poorly differentiated malignant tumor which appeared originally as Lymphoma in a mouse. It grows as both solid and ascitic forms [16]. The tumor implantation initiates a local inflammatory reaction, with increasing vascular permeability, which results in an intense ascitic fluid accumulation [17]. Ascitic fluid constitutes the direct nutritional source for tumor cells [18]. In untreated DLA tumor bearing mice, an increase in ascitic tumor volume was observed.

The toxic effects verified in this study against DLA and EAC cell lines might not be attributed to any of its specific constituents, but to the synergism between the cytotoxic effects of some components of the essential oil from the plant. Thus, it is possible to observe that the essential oil from flowers of *L. speciosa* presents an interesting biological activity concerning to its selective cytotoxic activity against DLA and EAC cell lines.

Monoterpene hydrocarbons like α -pinene and myrcene, an oxygenated monoterpene linalool; etc was earlier reported to possess cytotoxic effects against A-549 and HeLa cell lines [19] Tatman and Mo [20], verified that both linalool and α -pinene present cytotoxic activity against murine B16 melanoma and human HL-60 leukemia cells. Some other tested compounds, such as β -caryophyllene and α -humulene, showed high cytotoxic activity against this same cells [21,22] In fact, Sylvestre et al., [23] showed that myrcene is a low cytotoxic compound against DLD-1 cells. Sibanda et al [24] demonstrated that linalool is a compound with some antimicrobial activity, but not cytotoxic against SK-MEL-28, MDA-MB-231, MCF7, PC-3 and Hs 578T cells.

Isolation of active compounds and studies involving different cell lines and animal models might characterize the presence of other substances potentially active against several types of tumors and other therapeutic effects. The present study concludes that the active principles of the flower oil proved to have cytotoxic effect with the cancer cells. It is suggested that in future, the studies to be continued to find out the anticancer property of the oil using animal models.

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REFERENCES

1. Kakuda T, et al. Hypoglycemic effect of extracts from *Lagerstroemia speciosa* L. leaves in genetically diabetic KK-A^y mice. *Biosci Biotechnol Biochem.* 1996;60:204-208.
2. Hou W, et al. Triterpene acids isolated from *Lagerstroemia speciosa* leaves as alpha-glucosidase inhibitors. *Phytother. Res.* 2009;23:14-18.
3. Priya TT, et al. Amelioration of cisplatin induced nephrotoxicity in mice by an ethyl acetate extract of *Lagerstroemia speciosa* (L). *J Basic Clin Physiol Pharmacol.* 2007;18:289-298.
4. Priya TT, et al. Free radical scavenging and anti-inflammatory properties of *Lagerstroemia speciosa* (L). *Inflammopharmacol.* 2008;16:182-187.
5. Priya TT, et al. Hepatoprotective and free radical scavenging activities of *Lagerstroemia speciosa* Linn. leaf extract. *Orient Pharm Exp Med.* 2009;9:225-231.
6. Badami R and Kudari SM. Component acids of *Lagerstroemia speciosa* seed oil. *Indian. J Appl Chem.* 1970;33:213-215.
7. Jehan C, et al. A keto fatty acid from *Lagerstroemia speciosa* seed oil. *Phytochem.* 1990;29:2323-2324.
8. Laskarz S, et al. Extraction and chemical investigation of *Lagerstroemia speciosa* seed proteins. *Am Lab.* 1998;30:22-24.
9. Sinhababu A, et al. Characterization and identification of antibacterial components in extracts of seeds from *Lagerstroemia speciosa*. *Adv Food Sci.* 1999;21:19-22.
10. Guo Xiao-Ke. Synthesis and evaluation of gambogic acid derivatives as antitumor agents. Part III. *Chemistry & biodiversity.* 2013;10:73-85.
11. Okada Y, et al. A new triterpenoid isolated from *Lagerstroemia speciosa* (L.) Pers. *Chemical and Pharmaceutical Bulletin.* 2003;51:452-454.
12. Rahman MA, et al. Antinociceptive, antidiarrhoeal and cytotoxic activities of *Lagerstroemia speciosa* (L.) Pers. *Pharmacologyonline.* 2011;1:604-612.
13. NIST (National Institute of Standards and Technology). Mass Spectral Library (NIST/EPA/NIH) - 2005 Version.
14. Gupta SK. *Cytotoxicity Assays. A Handbook of practical and Clinical Immunology.* 2nd ed. CBS Publisher, New Delhi, India; 2002.
15. Segura JA, et al. Ehrlich ascites tumor unbalances splenic cell population reduces responsiveness of T cells to *Staphylococcus aureus* enterotoxin B stimulation. *Immunol Lett.* 2000;74:111-115.
16. Kleinsmith LJ. *Principles of Cancer Biology.* 4th ed. San Francisco: Pearson Benjamin Cummings, Michigan, United States; 2007.
17. Shimizu M, et al. Expression of cytosolic phospholipase A₂α in murine C12 cells, a variant of L929 cells, induces arachidonic acid release in response to phorbol myristate acetate and Ca²⁺ ionophores, but not to tumor necrosis factor-α. *J Pharmacol Sci.* 2004;96:324-332.
18. Fecchio D, et al. Studies on inflammatory response induced by Ehrlich tumor in mice peritoneal cavity. *Inflammation.* 1990;14:125-132.
19. Adams RP. *Identification of essential oil components by gas chromatography/ Mass Spectroscopy.* Allured Pubi Corp, Carol Stream, IL, USA; 1995.
20. Tatman D and Mo H. Volatile isoprenoid constituents of fruits, vegetables and herbs cumulatively suppress the proliferation of murine B16 melanoma and human HL-60 leukemia cells. *Cancer Letters.* 2002;175:129-139.
21. Robards K, Haddad PR, Jackson PE. *Principles and Practice of Modern Chromatographic Methods.* 1st Ed. Academic Press, New York, United States; 1994.
22. Luis da SS, et al. Cytotoxic evaluation of essential oil from *Zanthoxylum rhoifolium* Lam. leaves. *Acta Amaz.* 2007;37:281-286.
23. Sylvestre M et al. Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Guadeloupe. *J Ethnopharmacol.* 2006;103:99-102.
24. Sibanda S, et al. Composition and bioactivity of the leaf essential oil of *Heteropyxis dehniae* from Zimbabwe. *J Ethnopharmacology.* 2004;92:107-111.