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Research Article

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Phytochemical Screening and *In vitro* Evaluation of Anti-oxidant and Anti-arthritic Activity of Ethanolic Extract of Fruit-Seeds of *Lagerstroemia speciosa* L. (EE-FSLS)

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ABSTRACT

The objective of the present work is to evaluate *in vitro* anti-oxidant and anti-arthritic activity of fruits (with seeds) of *Lagerstroemia speciosa* L. Based on this, a new series of constituents had been planned to extract by ethanol from the fruit-seeds of *Lagerstroemia speciosa* L. The *in-vitro* anti oxidant activity was carried out by DPPH assay and the *in-vitro* anti arthritic activity was performed by BSA method. The results of DPPH free radical scavenging activity showed that the ethanolic extracts of fruit-seeds of *Lagerstroemia speciosa* (EE-FSLS) possessed good to moderate anti-oxidant activity. The percentage (%) inhibition at various concentration (10-250 µg/ml) of EE-FSLS was found to be 14.89%, 20.53%, 31.81%, 37.62%, 49.24% and 60.52% and the IC₅₀ value was found to be 152.78 µg/ml and the IC₅₀ value of Std. drug ascorbic acid was found to be 97.52 µg/ml. The *in-vitro* experimental data of BSA method displayed that EE-FSLS inhibit the protein denaturation and the percentage of protein denaturation by EE-FSLS was found to be 29.4%; 42.5% and 59.5% etc and executed good anti-arthritic activity.

Keywords: Bioactive compounds, Anti-oxidant, Anti-arthritic activity, DPPH, BSA, IC₅₀ etc.

ARTICLE INFO

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1. Introduction

The genus *Lagerstroemia* was first described by Carlos Linnaeus. 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 grows up to 10 meters high. Banaba has large green oblong leaves that are 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 [1, 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, lagerstroemin, 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) [4]. *Lagerstroemia speciosa* have been previously reported to have hypoglycemic activity by reducing fasting blood glucose of streptozotocin induced Diabetic rats. Apart from hypoglycemic activity [5-7] Banava leaves also possessed Antioxidant [8], Anti-inflammatory [9], Antiobesity [10], Antifibrotic [11]. 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 phyto-constituents and evaluate the in vitro cytotoxic activity of fruits (exo and endo carp) of *Lagerstroemia speciosa* L.

2. Materials and method

Chemicals and drugs: The all chemicals used for the extraction and phyto-chemical and biological screening were of LR and AR grade. Standard drugs ascorbic acid (antioxidant activity) and diclofenac sodium (anti-arthritis activity) were purchased from Local Retail Pharmacy Shop and solvents and other chemicals were used from Institutional Store and were of AR grade.

Methodology for extraction (Soxhlet Extraction) [12]

Introduction: When an organic substance is to be reserved from a solid, it is extracted by means of an organic solvent in which impurities are insoluble. In actual practice the International Journal of Pharmacy and Natural Medicines

extraction from solids is often tedious and requires through contact and heating with the solvent. This is done in a special apparatus, the Soxhlet Extractor. It consists of a glass cylinder having a side tube and siphon. The cylinder carries a water condenser at the top and is fitted below into the neck of a boiling round bottom flask.

Methodology:

First the dried fruits and seeds *Lagerstroemia speciosa* are triturate to make fine powder, the powdered material is placed into the thimble made of stout filter paper, and the apparatus is fitted up. The flask containing suitable solvent separately ethanol is heated on a water bath or on a heating mental. As the solvent boil, its vapors rise through the side tube up into the water condenser. The condensed liquid drops on the solid in the thimble, dissolves the organic substances present in the powdered material and filters out into the space between the thimble and the glass cylinder. As the level of liquid here rises, the solution flows through the siphon back into the boiling flask. The solvent is once again vaporized, leaving behind the extracted substance in the flask. In this way a continuous stream of pure solvent drops on the solid material, extract the soluble substance and returns to the flask. At the end of the operation the solvent in the boiling flask is distilled off, leaving the organic substance behind. Afterwards the ethanolic extract of fruit-seeds of *Lagerstroemia speciosa* (EE-FSLS) transfer in a clean and dried beaker and is concentrated by placing on a water bath and then cool, keep it in a freeze. From this concentrated extract the preliminary phytochemical screening has to be carried out.

Preliminary Phytochemical screening [13-20]

Preliminary phyto-chemical screening of EE-FSLS 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.

Biological Screening

(A) Evaluation of In vitro Anti-oxidant activity by DPPH Assay

Principle:

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assays evaluates free radical scavenging activity by measuring the color change that occurs when a DPPH radical is quenched by a free radical scavenger that donates a hydrogen atom.

Method [21]:

The ethanolic fruit-seeds extract was tested for the DPPH free radical scavenging activity according to the method of Pan et al. [22] with minor modification. 0.2 mL of the extract solution in ethanol (95 %) at different concentrations was added to 8 mL of 0.004 % (w/v) stock solution of DPPH in ethanol (95 %). The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm until the reaction reached the steady state, using a UV-Visible spectrophotometer. As a positive control, synthetic antioxidant gallic acid was used.

All determinations were performed in triplicate. The DPPH radical scavenging activity (S%) was calculated using the following equation:

$$S\% = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where A_{control} = absorbance of the blank control (containing all reagents except the extract solution) and A_{sample} = absorbance of the test sample.

(B) Evaluation of In vitro Anti-arthritis activity by BSA method [23-26]

Preparation of reagents: 5% Bovine serum albumin (BSA): Dissolved 5 g of BSA in 100 ml of water. Phosphate buffer saline pH 6.3: Dissolved 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na_2HPO_4), 0.24 g of potassium dihydrogen phosphate (KH_2PO_4) in 800 ml distilled water. The pH was adjusted to 6.3 using 1N HCl and make up the volume to 1000 ml with distilled water.

Method:

- Test solution (0.5 ml) consists of 0.45 ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution of various concentrations.
- Test control solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water.
- Product control (0.5 ml) consists of 0.45 ml of distilled water and 0.05 ml of test solution.
- Standard solution (0.5 ml) consists of 0.45 ml of Bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of Diclofenac sodium of various concentrations.

Various concentrations (100, 250, 500 $\mu\text{g/ml}$) of EE-FSLS and standard drug diclofenac sodium (100, 250, 500 $\mu\text{g/ml}$) were taken respectively. All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°C for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm. The control represents 100% protein denaturation. The results were compared with Diclofenac sodium. All determinations were done in triplicate. The percentage inhibition of protein denaturation can be calculated as:

$$\text{Percentage Inhibition} = 100 - [\{ (\text{optical density of test solution} - \text{optical Density of product control}) / \text{optical density of test control} \} \times 100]$$

3. Results and Discussion

(A) In vitro Antioxidant activity (DPPH free radical scavenging activity)

The DPPH radical scavenging activity of EE-FSLS was evaluated and compared with Ascorbic acid and the results are given in Table-1. The % inhibition at various concentration (10-250 $\mu\text{g/ml}$) of EE-FSLS as well as standard Ascorbic acid (4 -50 $\mu\text{g/ml}$) were shown in Fig 1 and 2. The IC_{50} values are calculated from graph and were found to be 97.52 $\mu\text{g/ml}$ (Ascorbic acid) and 152.78 $\mu\text{g/ml}$ (EE-FSLS).

Table 1: Results of DPPH scavenging activity

Sl. No.	Concentration (ug/mL)	Absorbance (A)	S% = [(A ₀ -A)÷A ₀] X 100	IC ₅₀ (ug/ml)
Control (DPPH Sol.)				
1.	0.1 mM in ethanol	1.188 (A ₀)		-
Std (Ascorbic Acid)				
1	4	0.981	17.42	97.52
2	6	0.959	19.23	
3	8	0.915	22.97	
4	10	0.873	26.51	
5	25	0.555	53.28	
6	50	0.033	97.22	
EE-FSLS				
1	10	1.011	14.89	152.78
2	25	0.944	20.53	
3	50	0.810	1.81	
4	75	0.741	37.62	
5	100	0.603	49.24	
6	250	0.469	60.52	

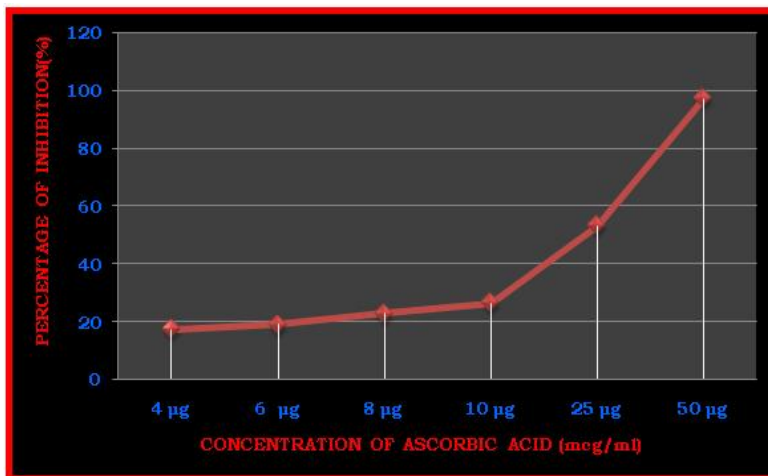


Figure 1: DDPH scavenging activity of Ascorbic acid

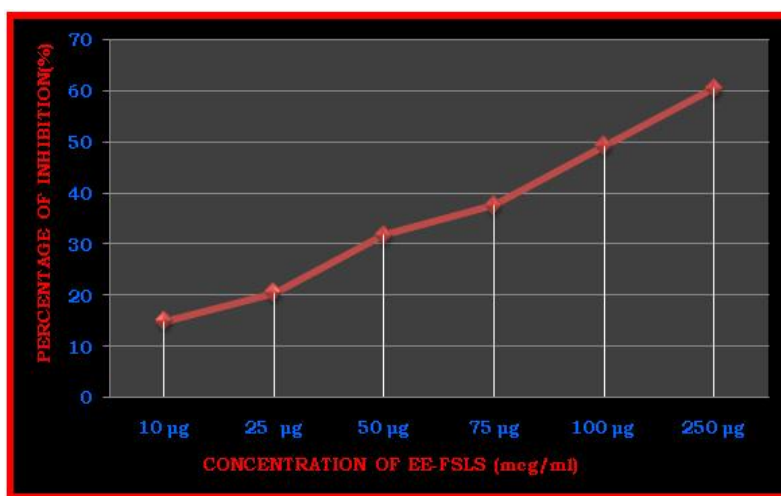


Figure 2: DDPH scavenging activity of EE-FSLS

(B) In vitro Anti arthritic activity

The *In vitro* anti-arthritic activity of EE-FSLS was evaluated by Bovine serum albumin denaturation method and the

experimental results were given in Table-2 and the percentage of inhibition of EE-FSLS were compared with standard drug diclofenac sodium which was shown by Fig: 3.

Table 2: Result of *In-vitro* anti-arthritic activity of drugs in BSA denaturation method

Drug	Conc. (µg/ml)	% inhibition
Test Control	-	-
Product Control	-	-
EE- FSLS	100	29.4±2.3
	250	42.5±1.2
	500	59.5±2.7
Diclofenac sodium	100	67.3±1.3
	250	77.4±2.5
	500	89.7±1.3

Values are in Mean ± SD, n=3.

Denaturation of protein is the main cause of rheumatoid arthritis was well documented. Production of autoantigen in arthritis mainly due to the denaturation of protein. The inhibition of denaturation of protein is one of the possible targets for the treatment of arthritis. In present International Journal of Pharmacy and Natural Medicines

study displays that the EE-FSLS inhibit the protein denaturation and can be used as anti-arthritic agent. The percentage of protein denaturation by EE-FSLS have been found 29.4%; 42.5% and 59.5% etc.

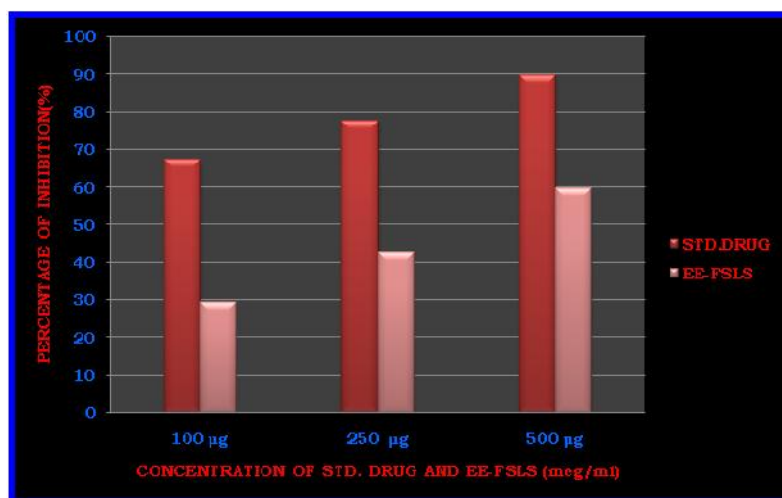


Figure 3: *In-vitro* anti-arthritic activity of Std. drug diclofenac sodium and EE-FSLS in BSA denaturation method.

4. Conclusion

The results obtained from the *in-vitro* studies performed by using DPH Assay method displayed that the EE-FSLS exhibited good antioxidant activity with reference to standard drug ascorbic acid and as well as the results obtained from the *in-vitro* studies performed by using BSA had shown that EE-FSLS exhibited good anti-arthritic activity. So finally it can be concluded that EE-FSLS can be used in the treatment of both cell damage caused by free radicals and rheumatoid arthritis.

5. References

- [1] "Lagerstroemia speciosa (L.) Pers. pride of India." PLANTS Profile, United States Department of Agriculture / Natural Resources Conservation Service. **2008**.
- [2] Eduardo B. Principe and Aurora S. Jose (2002). "Propagation Management of Herbal and Medicinal Plants". Research Information Series on Ecosystems 14 (2).
- [3] Tomonori U, Akio S, Takami K, xanthine oxidase inhibitors from the leaves of Lagerstroemia speciosa (L.). Pers. Journal of Ethno pharmacology. **2004**; 93: 391-395.
- [4] Custer C, Deocaris et al. Hypoglycemic activity of Irradiated Banaba Lagerstroemia speciosa Linn. Leaves. Journal of Applied Sciences research **2005**, 1-1: 95-98.
- [5] Amornnat Thuppia, et al. The Hypoglycemic effect of water extract from the leaves of Lagerstroemia speciosa in streptozocin induced Diabetic rats. Journal of Science and Technology. Apr **2009**; 31-2:133-137.
- [6] Tomonori U, et al. Anti oxidative activity of water extracts of Lagerstroemia speciosa leaves. Bioscience, Biotech, Biochem. **1997**, 61-10: 391-395.
- [7] Priya TT. Sabu Mc, Jolly C. Free radical scavenging and Anti inflammatory properties of Lagerstroemia speciosa. Inflammatory Pharmacology. **2008**, 16: 128-187.
- [8] Guy Klein, et al. Anti diabetes and anti obesity Activity of Lagerstroemia speciosa. Ecam Advance Access. **2007**; 14: 1.
- [9] Vinoth Prabhu, et al. 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] Arun Bhal, B.S Bhal. Advanced Organic Chemistry, First Edition 2010, pp 13.
- [11] U. Satyanarayana, U. Chakrapani, Bio-chemistry, 3rd edition, PP- 9-10, 43-44.
- [12] P.C Dandiya, P.K. Sharma, Bio-chemistry and clinical pathology, second edition, pp. 17-18, 24, 47-48.
- [13] MN Chatterjea, Rana Shinde, Text Book of Medical Biochemistry, 7th edition, pp.74-76.
- [14] C.K. Kokate, A.P. Purohit, S.B. Gokhale, Pharmacognosy, 42 Edition, pp- A.1
- [15] Jaswant Kaur, PV Chemistry of Natural Products, **2010** edition, PP-113-114, 116, 344-346, 381.
- [16] Jaswant Kaur, PV Chemistry of Natural Products, **2010** edition, PP-113-114, 116, 344-346, 381.
- [17] Dr. G. Devala Rao, A Manual of Practical Biochemistry, pp 17.
- [18] Gurdeep R. Chatwal, Organic Chemistry of Natural Products, Vol-II, pp 4.1-4.2.
- [19] Nagendrappa CG. An appreciation of free radical chemistry-3, free radicals in diseases and health, Resonance. **2005**; 10: 65-73.
- [20] Madan Singh, Prashant Soni, Neeraj Upmanyu and Yogesh Shivhare. In-vitro Anti-arthritic Activity of Manilkara zapota Linn. Asian J. Pharm. Tech. **2011**, 1(4): 123-124.
- [21] Deshpande V, Jadhav VM and Kadam VJ. In-vitro antiarthritic activity of Abutilon indicum (Linn.) Sweet. J. of Pharm. Res. **2009**, 2(4): 644-645.
- [22] Seema Chaitanya Chippada and Meena Vangalapati. Antioxidant, an anti-inflammatory and anti-arthritic activity of Centella asiatica extracts. J. Chem. Bio. Phy. Sci. **2011**, 11(2): B, 260– 269.

- [23] Sharan Suresh Volluri, Srinivasa Rao Bammidi, Seema Chaitanya Chippada and Meena Vangalapat. In-Vitro Anti-Arthritic Activity of Methanolic Extract of *Bacopa Monniera*. International Journal of Chemical, Environmental and Pharmaceutical Research. **2011**, 2(2-3): 156-159.
- [24] Kujala TS, Loponen JM, Klika KD, Pihlaja K, Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effect of cold storage on the content of total phenolics and three individual compounds, J Agric Food Chem, **2000**, 48: 5338–5342.