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Short Communication

EXTRACTION, ISOLATION AND STRUCTURAL ELUCIDATION OF BIOACTIVE COMPOUND FROM THE LEAVES EXTRACT OF *LAGERSTROEMIA PARVIFLORA*

Namita Bharadwaj[1], Anjna Chaturvedi*[2]

[1]*Dr. C. V. Raman University, Kota Bilaspur, Chattisgarh, India*
[2]*Research scholar, Dr. C. V. Raman University, Kota Bilaspur, Chattisgarh, India*
**Corresponding author: manojkorba.aisect@gmail.com*

ABSTRACT

For thousands of years, natural remedies were the only choice for the prevention and treatment of human diseases. Drug development relies heavily on natural products. Natural remedies always have small amount of bioactive natural ingredients. Today, developing effective and selective strategies for extracting and isolating those bioactive natural compounds is critical. *Lagerstroemia parviflora* (*L. parviflora)* (Lythraceae) commonly known as Landia in India and Seja in Bundelkhand; is a species widely distributed in almost all moist and dry deciduous tracts of India. In view of its wide distribution, the tree can withstand great variation in climate. The current investigation involves the isolation, structure characterization and prediction of antioxidant compounds from the leaves of *L.parviflora*. The leaves samples were used for the extraction of plant constituents with methanol by using maceration method. The isolated pure compounds were characterized as (2*S*)-5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one by ^{1}H NMR, IR and MASS spectroscopy. The pure compounds obtained from methanol extract of the selected plant species could be a good source of natural antioxidant.

Keywords: Natural remedies, *Lagerstroemia parviflora,* Structure elucidation,Chromatography

1. INTRODUCTION

Because of the unrivaled abundance of chemical variety, natural products, such as plant extracts, either as pure chemicals or as standardized extracts, give limitless prospects for new drug discoveries [1]. According to the World Health Organization (WHO), more than 80 % of the world's population relies on traditional medicine for their primary healthcare requirements. In Asia, the use of herbal remedies reflects a long history of human interactions with the natural world. Traditional medicinal plants have a variety of chemicals that can be utilized to treat both chronic and infectious disorders [2].

There are a number of crude drugs where the plant source has not yet been scientifically identified. A phytochemical is a natural bioactive compound found in plants foods that works with nutrients and dietary fiber to protect against diseases. Many researchers suggest that, phytochemical works together with nutrients found in fruits, vegetables and nuts. They can have complementary and overlapping mechanism of action in the body including antioxidant effect.

L. parviflora (Lythraceae), commonly known as Landia in India and Seja in Bundel khand; is a species widely distributed in almost all moist and dry deciduous tracts of India. In view of its wide distribution, the tree can withstand great variation in climate. It is often found as a companion to natural sal and teak it occurs as a distinct species in forests of sub- Himalayan tracts, Assam, Madhya Pradesh, Orissa, Maharashtra, Gujarat, Andhra Pradesh, Karnataka and Tamil Nadu (except Nilgiris and arid regions). In Madhya Pradesh, it is common in all districts [3].

The tree is primarily use for timber, the bark of *L. parviflora* contain tannin (7%-10%) and is used locally for tanning and dyeing lather and for dyeing cotton thread. It also has some medicinal importance [4]. Mazumder et al. reported the antibacterial activities of the leaves of the plant [5] and Bhakuni et al. reported the antiasthmatic activity of the flowers of *L. parviflora* [6]. Mazumder et al. reported antipyretic potential of *L. parviflora* leaves [7]. The leaf juice of this plant is used in traditional medicine to treat fever in Jharkhand, India [8].

In past, the plant or microbial extracts in crude or partially-purified forms were the only sources of medication available for the treatment of human and animal diseases. This gave an idea that the effect of a drug

in human body is due to an interaction of drug with biological molecules. This opened new doors in pharmacology, as pure, isolated chemicals, instead of extracts, as the standard for the treatment of diseases. At present, there are innumerable number of such bioactive compounds isolated form crude extracts and their chemical structure were elucidated [9]. Moreover, plants have always been a source of a wide array of secondary metabolites with potential pharmacological properties [10]. Polyphenolic (flavonoids) compounds occur ubiquitously in foods of plant origin have many beneficial health effects due to their potential anti-oxidant, anti-inflammatory and cancer-preventive activities [11]. Therefore, the objective of this study was to isolation of biologically active plant secondary metabolites using column-chromatographic techniques from *Lagerstroemia parviflora.*

2. MATERIAL AND METHODS

The solvents like methanol was used in this experiment obtained from HiMedia Pvt. ltd., Prepared TLC (Aluminum TLC Silica Gel 60F254 Plates) were purchased from Merck India Pvt. Ltd., other chemicals were usedanalytical grade. All glassware used in this experiment was from borosil, India.

2.1. Defatting of plant material

Powdered leaves of L. parviflora were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place [12].

2.2. Extraction by maceration process

A 89gm of dried plant material were extracted with methanol using maceration method for 48 hrs. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [12].

2.3. Selection and optimization of mobile phase by Thin Layer Chromatography

The adsorption phenomenon is the basis for thin-layer chromatography. The mobile phase, which contains the dissolved solutes, travels across the stationary phase's surface in this form of chromatography.

For this, extract under examination was diluted with respective solvent. Extract was then subjected to thin-layer chromatography (TLC) as per conventional one-dimensional ascending method using commercially available silica gel sheets 60F254, 7X6 cm (Merck) which were cut into thin plates with an ordinary household scissor. A soft pencil was used to make plate marks. The sample was spotted on TLC plates using glass capillaries. With the various solvent system employed, a sample volume of about 2 µl was applied via capillary at a distance of 1 cm at the bottom of twin trough chamber [13].

2.4. Solvent system used for column chromatography

Chambers were utilized for chromatograph development after a 20-minute pre-saturation with mobile phase. Plates were dried after the run and examined under UV light for the detection of bands or spots. The retention factor (R_f) values determined for distinct samples were used to express the active compound's mobility.

Table 1: Solvent system used for column chromatography

Mobile phase	Composition (v/v)
Toluene: Ethyl acetate	9:1
Toluene: Ethyl acetate	8:2
Toluene: Ethyl acetate	7:3
Chloroform: Methanol	9:1
Ethyl acetate: Methanol	5:5

2.5. Detection and Calculation of R_f Value

Once the chromatogram was developed, the Rf Value of the spots were calculated using the formula:

Rf=(Distance travelded by solute)/(Distance travelded by solvent front).

2.6. Column specification

Table 2: Column specification

Column size	Glass Coloum, 100 x 3 Cms
Stationary Phase	Silica for column (60 to 120 mesh size)
Elution mode	Isocratic
Mobile Phase	Toluene: Ethyl Acetate (7:3 v/v)
Extract	Methanolic extract of *Lagerstroemia parviflora*
Visualized by	Short UV (254nm), long UV (365nm) and Normal light
Identification of similar fractions	UV vis. spectroscopy and Visualized by Short UV (254nm), long UV (365nm) and Normal light

2.7. Preparation of column

Column packing was done by wet packing method. Silica gel activated at 105°C was taken and suspended in the mobile phase composed of Toluene: Ethyl Acetate, 7:3 v/v. It was then transferred in a column and allowed to settle down. At the top of the silica layer, a cotton plug was kept to avoid disturbance in the silica layer during elution.

2.7.1. Preparation of sample

A 5gm of methanolic extract was taken in a beaker; to it, silica gel used for column chromatography (60-120 mesh size) and a sufficient amount of mobile phase was added. The slurry was made and introduced from the top of the silica gel column over the cotton plug.

2.7.2. Isocratic elution technique

In order to fractionate the components of isocratic elution technique, a mobile phase which was composed of Toluene: Ethyl Acetate (7:3) was used for elution. The 10ml of each fraction was collected and the solvent was recovered by distillation. The fractions were collected, concentrated, stored and subjected to TLC.

2.7.3. Identification of similar fractions

The 110 fractions of column chromatographic elution were monitored by TLC Toluene: Ethyl Acetate (7:3); using UV chamber at short UV and long UV light and treated with specific reagent for the identification of single isolated compound. The fractions which show similar fingerprint profile on TLC were collected and mixed. Fractions which showed a single compound and have similar Rf value were dried and the compound was purified by recrystallization procedure [14].

2.8. Characterization of isolated compound

The chemical test with lead acetate was used to define pooled fractions that had the same fingerprint profile on TLC. The presence of flavonoids was verified by the formation of a yellow-colored precipitate. Recrystallization was used to dry and purify the fraction(s). For structural elucidation, the isolated chemical was exposed to UV, IR, NMR, and Mass spectroscopy.

2.9. UV-visible spectroscopy

For qualitative investigation and identification of specific groups of chemicals in pure and biological mixtures, UV-visible spectroscopy can be used. Because aromatic compounds are strong chromophores in the UV region, UV-visible spectroscopy is used for quantitative investigation. UV-visible spectroscopy can be used to identify natural substances. Furthermore, it was discovered that spectroscopic UV-Vis methods are less selective and just provide information on the composition of the overall polyphenol content. When compared to other procedures, this one takes less time and is less expensive [9]. The UV absorption spectrum of compound was recorded in the range of 200-400nm on (LABINDIA 3000 +) UV spectrophotometer at 1 cm path length.

2.10. Infra red spectroscopic Analysis

Infrared spectroscopy using a Fourier transform is a useful technique for identifying functional groups in plant extracts. It aids in molecular identification and structural determination. It's a high-resolution analytical technique for determining chemical components and structural compounds. FTIR may be used to fingerprint plant extracts or powders in a quick and non-destructive manner [10]. The IR spectrum of compounds was recorded on Bruker Alpha using the solid plate technique with KBr.

2.11. NMR Analysis

Magnetic Resonance of Nuclei Physical, chemical, and biological characteristics of matter are determined through spectroscopy. The one-dimensional method is often utilized, however two-dimensional NMR techniques might be applied to produce the complex structure of the molecules. The molecular structure of solids is determined via solid-state NMR spectroscopy. 1H-NMR is used to find out the number of hydrogen are present in the compound and to find out how the hydrogen atoms are connected [15]. ^{1}HNMR was recorded on Bruker DRX -300 (300 MHz FT-NMR) in $CDCl_3$ using TMS as an internal standard.

2.12. Mass Analysis

Mass spectrometry is a strong analytical technique for determining the structure and chemical characteristics of molecules, as well as identifying new chemicals and quantifying known ones. The molecular weight of the sample may be determined using the MS spectrum. This method is commonly used for elucidating the structure of organic compounds, peptide or oligonucleotide sequencing, and monitoring the presence of previously characterized compounds in complex mixtures with high specificity by simultaneously defining the molecular

weight and a diagnostic fragment of the molecule The MS spectra were performed using AB SCIEX 3200 QTRAP® LC/MS/MS System. Equipped with fused silica capillary column (30 m× 0.25 i. d. film thickness 0.25μm). Injector and mass transfer line temperature were set at 250 and 300ºC, respectively. [16].

3. RESULTS AND DISCUSSION

The different mobile phases were tried for optimization of column chromatography like Toluene: Ethyl acetate (9:1), Toluene: Ethyl acetate (8:2), Toluene: Ethyl acetate (7:3), Chloroform: Methanol (9:1) and Ethyl acetate: Methanol (5:5). On the basis of proper separation and resolution, Toluene: Ethyl acetate (7:3) was selected as optimized mobile phase. In comparison to other mobile phases, Toluene: Ethyl acetate (7:3) produces a large number of spots with high resolution in normal, short, and long UV light. Total eight spots were found at Rf value 0.06, 0.21, 0.46, 0.58, 0.65, 0.71 in normal light, short UV and long UV light. The fractions with a single spot under UV light and a comparable Rf value were gathered and combined to produce a pooled fraction. UV, IR, NMR, and MASS spectroscopy were utilized to characterize isolated chemicals using these pooled fractions. The UV spectra of isolated compound showed the maximum absorbance at 294 nm, The different starching and banding position were found at C-O (str.)-1079. 5614, C=C (str.)-1423.7035, C=O (str.)- 1658.3458, C-H(str.)- 2899.3430, O-H(str.)- 3343.8778 in FT-IR Interpretation. The NMR peaks of isolated fraction showed at δ: 3.13 (m, 1H, CH_2), 3.38 (m, 1H, CH_2), 5.35 (s, 3H, OH), 5.09 (s, 1H, CH), 5.18 (s, 1H, CH), 6.18(t, 1H, CH), 6.68 (d, 2H, CH), 7.19(d, 2H, CH) and ESI-MS (m/z): 270 (M+2). The probable interpretation on the basis of above data the compound was found (2*S*)-5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one a flavonoid compound.

Table 3: Characterization of Isolated compound

No. of fractions	TLC UV spectra		Chemical Test
	UV-254	UV-365	Aluminium chloride test
1-15	No Spot	No Spot	-Ve
16-28	2 Spot	2 Spot	-Ve
29-36	2 Spot	3 Spot	-Ve
37-45	**1 Spot**	**1 Spot**	+Ve
46-55	3 Spot	5 Spot	-Ve
56-63	1 Spot	2 Spot	-Ve
64-75	2 Spot	3 Spot	-Ve
76-83	1 Spot	2 spot	-Ve
84-92	2 Spot	3 Spot	-Ve
93-102	2 Spot	3 Spot	-Ve
102-106	1 Spot	2 Spot	-Ve
106-110	No Spot	No Spot	-Ve

The fractions with a single spot under UV light and a comparable Rf value were gathered and combined to produce a pooled fraction. Isolated chemicals were characterized using these pooled fractions.

Table 4: Interpreted data of different spectra of isolated compound (37-45)

Method	Spectral interpretation
UV (λ_{max})	294 nm
IR	C-O (str.)- 1079.5614, C=C (str.)- 1423.7035, C=O(str.)- 1658.3458, C-H(str.)- 2899.3430, O-H(str.)- 3343.8778
^{1}H NMR (MEOD-500Mz)	δ: 3.13 (m, 1H, CH_2), 3.38 (m, 1H, CH_2), 5.35 (s, 3H, OH), 5.09 (s, 1H, CH), 5.18 (s, 1H, CH), 6.18(t, 1H, CH), 6.68 (d, 2H, CH), 7.19(d, 2H, CH).
ESI-MS (m/z)	270 (M+2)

Predicted Structure

IUPAC Name	(2*S*)-5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one

4. CONCLUSION

The current investigation involves the isolation, structure characterization and prediction of antioxidant compound from the leaves of *L. parviflora*. The leaves samples were used for the extraction of plant constituents with methanol by using maceration method. The isolated pure compounds were characterized as (2*S*)-5,7-dihydroxy-2-(4-hydroxyphenyl)-

2,3-dihydrochromen-4-one by ^{1}H NMR, IR and MASS spectroscopy. The presence of trihydroxyflavanone that is flavanone substituted by hydroxyl groups at positions 5, 6 and 4' in *L. parviflora* could possibly give potent antioxidant activity and the plant and isolated compound can be use as various pharmacological activities. The pure compounds obtained from methanol extract of the selected plant species could be a good source of natural antioxidant.

5. REFERENCES

1. Cosa P, Vlietinck A J, Berghe D V, Maes L. *J Ethnopharmacol.,* 2006; **106:**290-302.
2. Duraipandiyan V, Ayyanar M, Ignacimuthu S. *BMC Complementary Altern Med.,* 2006; **6:**35-41.
3. Singh S. Safe guard against introduction of against introduction of dangerous pathogens on seed of toxic trees. Proc. Nath. Seminar on tree seeds. Hyderabad, 1986, pp.147-153.
4. Anonymous. The wealth of India, raw material, Vol. 3. D-E, New Delhi: Council of Scientific and Industrial Research 1952.
5. Mazumder A, Saha BP, Basu SP, Mazumder R. *Indian J Nat Prod.,* 2003; **19:**20-24.
6. Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN. *Ind J Exp Biol.,* 1969; **7:**250-256.
7. Mazumder A, Saha BP, Basu SP, Mazumder R. *Pharm Biol.,* 2005; **43:**64-66.
8. Jain SK, Tarafdar CR. *Econ Bot.,* 1970; **24:**241-243.
9. Bajpai VK, Kang SC. *J Food Safety*, 2011; **31:**276-283.
10. Russell W, Duthie G. *Proc Nutr Soc.,* 2011; **70:** 389-396.
11. Li AN, Li S, Zhang YJ, Xu XR, Chen YM, Li HB. *Nutrients,* 2014; **6:**6020-6047.
12. Sharma S, Jain PK, Parkhe G. *Journal of Drug Delivery and Therapeutics,* 2020; **10(3-s):**86-90.
13. Hamburger MO, Cordell GA. *J Nat Prod.,* 1987; **50:**19-22.
14. Asila AS Al H, Mohammad AH. *Egyptian Journal of Basic and Applied Sciences,* 2018; **5(2):**157-164.