



## Research Article

### ISOLATION AND CHARACTERIZATION OF GALLIC ACID FROM THE ETHANOLIC EXTRACT OF LEAVES OF *BUTEA MONOSPERMA* (LAMB)

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#### ABSTRACT

*Butea monosperma* (Lamb) (Apocynaceae) popularly known as Palash in Tamil contains a number of phytoconstituents viz phytosterols, glycosides, alkaloids, proteins, phenolic compounds, amino acids, flavonoids and terpenoids. The objective of the present study was to isolate and characterize phytoconstituents from the ethanolic extract of leaves of *Butea monosperma* (Lamb). Ethanolic extract was subjected to column chromatography and eluted mixtures of increasing order of polarity composed of chloroform: methanol to isolate phytoconstituents. Gallic acid was isolated from the ethanolic extract of the leaves of the plant. The yield of compound was 0.062% w/w. m.p 258°C to 260°C  $\lambda$  max in EtOH: 272nm, R<sub>f</sub> value 0.4 in 0.83.Ethyl formate: Dichloromethane: Formic acid: Acetic acid (5:5:2:2). The structure of the isolated compound was established on the basis of elemental analysis and spectroscopic evidences (IR, UV, <sup>1</sup>H NMR, CNMR, MS) the compound was concluded as Gallic acid. *Butea monosperma* contains gallic acid which may be responsible for various pharmacological activities of the plant.

**KEYWORDS:** *Butea monosperma*, Gallic acid, Ethanolic extract, Isolation, Leaves.

#### INTRODUCTION

*Butea monosperma* (L) (Apocynaceae) species are growing widely in India. It is a deciduous tree widely distributed in common rather moist garden in lawns and in open plantation<sup>1</sup>. It grows as spreading shrub or small tree to a height of 7-8m [20-25 feet]. It contains a number of phytoconstituents viz alkaloids, phytosterols, amino acids, glycosides, proteins, phenolic compounds and flavonoids etc. Reported pharmacological activities of *Butea monosperma* plant are antifungal,<sup>2</sup> antiinflammatory<sup>3</sup>, antimicrobial<sup>4</sup>, anticonvulsive<sup>5</sup>, antifertility,<sup>6</sup> antidiarrheal activity<sup>7</sup> and anti cancer activity.<sup>8</sup> Herbal medicine is still the dependence of about 75–80% of the world population, mainly in the growing countries, for primary health care because of good acceptability, better compatibility with the human body and minimal side effects. Natural products play an important role in the discovery of new potential bio active molecules, which are clinically used to eradicate ailments and diseases. Medicinal plants provide biomolecules and precursors for the development of novel synthetic or semi-synthetic drug molecules. Chemical synthesis of derivatives of the isolated lead natural compounds are done by implementing different synthetic tools and it will serve as a promising line for lead generation. Variety of reasons has been cited for the need for studying medicinal plants. Most of the traditional knowledge about medicinal plants was in the form of oral knowledge that had been lost with persistent invasions and cultural adaptations. There was no uniform or standard procedure for maintaining the inventory of these plants and the knowledge about their medicinal properties. There is a prevalence of using plants and plant based products in various contemporary and traditional systems of medicines, without any written documentation or regulation. Therefore, it is essential that such uses of natural products be documented and studied for systematic regulation and wide-spread application. The leads for a significant number of modern synthetic drugs have originated from isolated plant ingredients, as the search for newer entities

begins from existing drugs or from traditional medicinal systems. The leaves of *B.monosperma* plant are used in the treatment of tumorous hemorrhoids.<sup>9</sup> The literature review revealed that it contains alkaloids, protein, terpenoids, flavonoids, phenolic compounds and minerals.<sup>10</sup> The present study deals with isolation and characterization of Gallic acid from ethanolic extracts of leaves of *B. monosperma* (L).

#### MATERIALS AND METHOD

##### Collection and authentication of the plant material

*B.monosperma* leaves were collected from and Shervaroyan hills, Tamil Nadu, India. and authenticated by Dr.A.Balasubramanian (ABS Botanical garden, Karipatty, Salem) (voucher specimen No PARC/2008/241) the leaves were separated from the plant and dried under shade.

##### Extraction and isolation of the compound

The shade dried and coarsely powdered leaves were extracted with petroleum ether, chloroform, acetone and ethanol by using soxhlet apparatus. Ethanol extract was subjected to column chromatography by using silica gel and eluted with solvent mixture of increasing order of polarity, composed of petroleum ether, acetone and chloroform and ethanol. All the fractions were observed on TLC. Fractions were collected with Ethyl formate: Dichloromethane: Formic acid: Acetic acid (5:5:2:2) *Butea monosperma* were pulled together as these fractions showed as a single spot of same R<sub>f</sub> value in TLC. It was dehumidified in a water bath. The residue was dissolved in a mixture of chloroform: methanol (60:40) with little warming on a water bath. It was kept in refrigerator, when yellow needle shaped crystals of Gallic acid were obtained. The structure of the isolated compound was established on the basis of elemental analysis and spectroscopic evidences (IR, UV, <sup>1</sup>H NMR, <sup>13</sup>C NMR, MS) the structure was

programmed to obtain the chemical shifts of both proton and carbon.

## RESULTS

### Characterization of the compound

Phytochemical analysis (Ferric chloride test) of the compound confirms its phenolic in nature. Based upon the number of the formula could be tentatively CHO from  $^{13}\text{C}$  NMR and  $^1\text{H}$  NMR the number of carbon H was found to be near to the first formula i.e. C H O since the compound gives positive test for flavonoids. The elemental analysis (Elemental vario EL 111) revealed that the compound contains 76.36% of C, 12.36% of H and 10.86% of O. The N% was found to be nil. Now base on the number of O (4or5) in the proposed compound the molecular weight could be 136 respecting based upon the number of the formula could be tentatively CHO from  $^{13}\text{C}$  NMR and  $^1\text{H}$  NMR the number of carbon H was found to be near to the first formula i.e. C H O since the compound gives positive test for flavonoids. Based on the functional group analysis it was found that the nature of oxygen was hydroxyl, also supported by IR spectroscopy. (Perkin-Elmer IR spectrometer)

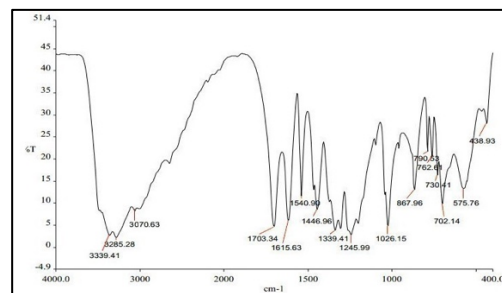


Fig 2: IR spectrum of compound

Table 1: FT-IR spectra interpretation of compound

Sr. No.	IR Peaks (cm <sup>-1</sup> )	Interpretation
1	3339.41	O-H ,stretching
2	3285.28	broad, O-H
3	3070.63	C-H (stretch,Ar)
4	1615.63,1540.90, 1446.96	C=C ( stretch,Ar)
5	1245.99	O-H (bend,Phe)
6	1703.34	C=O

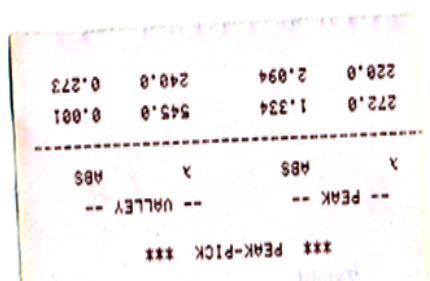
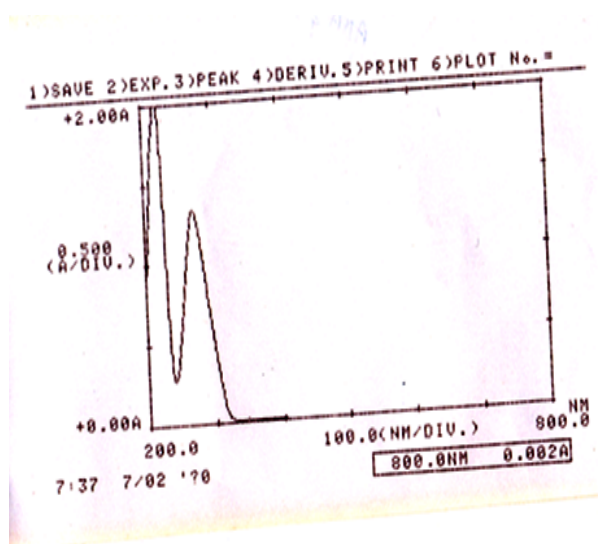


Fig 1: UV Spectra of compound

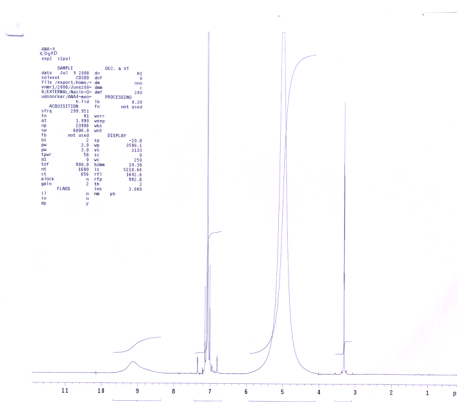


Fig 3a:  $^1\text{H}$  NMR spectrum of compound

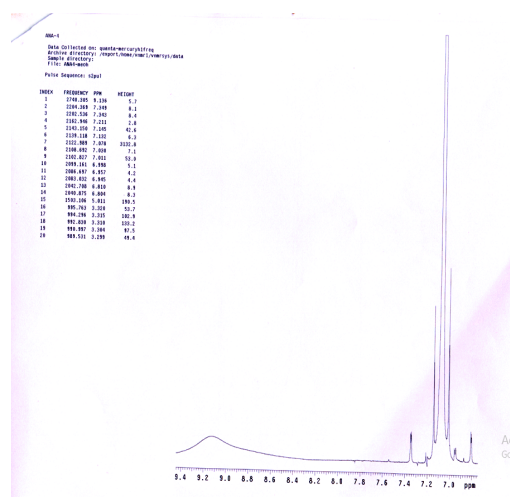
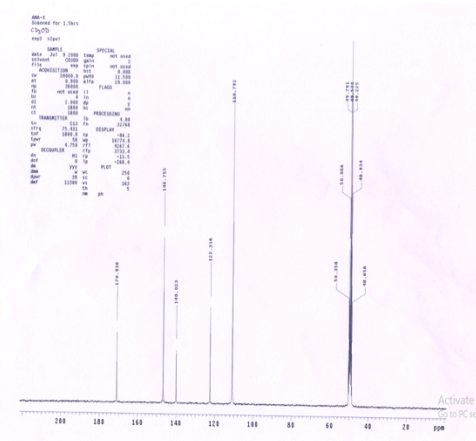


Fig 3b:  $^1\text{H}$  NMR spectra of compound

Table 2: Interpretation data of  $^1\text{H}$  NMR spectra

Sr. no.	$\delta$ ppm (Chemical shift)	Proton type
1	9.13, singlet	Ar-COO-H
2	7.07, triplet	Ar-H
3	5.01, singlet	Ar-O-H
4	3.31, multiplet	Solvent peak ( $\text{d}_4\text{-CH}_3\text{OH}$ )

Fig 4:  $^{13}\text{C}$  NMR Spectrum of Gallic acidTable 3: Interpretation data of  $^{13}\text{C}$  NMR spectra

Sr. no.	$\delta$ ppm (Chemical shift)	Carbon type
1	170.93, singlet	C=O (Acid)
2	146.75, singlet	-C- (Ar-para OH)
3	140.02, singlet	Ar-C
4	122.79, singlet	-C- (Ar-COOH)
5	110.79, singlet	-C- (Ar-meta OH)
6	49.50, septet	Solvent peak ( $\text{d}_4\text{-CH}_3\text{OH}$ )

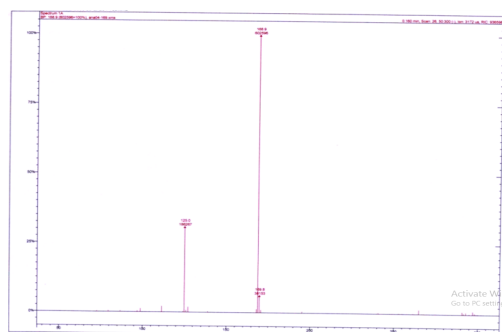


Fig 5: Mass Spectrum of compounds

Table 4: Interpretation data of Mass spectra of compounds

S.No	m/e ratio	Peak intensity (%)	Fragment type
1	125	40	$\text{C}_6\text{H}_5\text{O}^+$
2	168.9	100	$\text{C}_6\text{H}_5\text{O}^+$
3	169.9	5	$\text{C}_6\text{H}_6\text{O}^+$

## DISCUSSION

The compound is yellow crystalline compound melting point  $258\text{--}260^\circ\text{C}$ . (Fig.1)  $\lambda_{\text{max}} = 272\text{nm}$ . (Fig.2) IR absorptions band appears at  $3339.41\text{ cm}^{-1}$  OH (Str),  $3285\text{ cm}^{-1}$  OH (Broad),  $1615\text{ cm}^{-1}$  C=C (Str, Ar),  $1703.34\text{ cm}^{-1}$  C=O. (Fig.3) NMR spectrum of this compound having 9.13 singlets (Ar COOH), 7.07 triplets (Ar-H), 5.01 singlet (Ar-OH), 3.31-multiplet solvent peak. The carbon of 170.93 singlet C=O Acid, 146.75 singlet -C- (Ar-para OH), 140.02 singlet Ar-C, 122.79 singlet -C- (Ar-COOH). (Fig.4). Mass

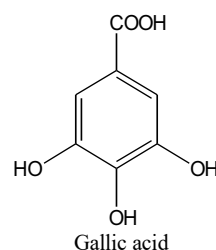
spectra of this compound suggested that its molecular formula  $169.9\text{ MF} - \text{C}_7\text{H}_6\text{O}_5$  having characteristic fragments. Mass spectra of this compound suggested that its molecular formula  $169.9\text{ MF} - \text{C}_7\text{H}_6\text{O}_5$  having characteristic fragments. Mass spectrum observed at  $m/z$  125, 168.9, 169.9. NMR spectrum of this compound based on the melting point and other related data (IR, NMR and Mass) the structure of the isolated compound was proposed as Gallic acid.

**Chemical name of the isolated compound;** 3, 4, 5 Tri hydroxy benzoic acid

**Molecular formula:**  $\text{C}_7\text{H}_6\text{O}_5$

**Molecular weight:** 170.12g/mol

**Proposed structure of Gallic acid**



## CONCLUSION

Gallic acid was isolated and characterized from ethanol extract of *Butea monosperma* leaves and this is a flavonoid. Furthermore, flavonoids have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis. Gallic acid reduces Enrich ascites carcinoma induced in albino mice. Gallic acid as a selective anticancer agent that induces apoptosis in human hepato cellular carcinoma cells. Further this study proposed to evaluate that the Gallic acid is responsible for anticancer activity of this plant.

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