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# Isolation, Characterization and Phytochemical Evaluation of Active Compound Thymol from *Cyclea Peltata* (Lam)

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**Abstract**

*Cycleapeltata*(Lam) belonging to the family Menispermaceae is a common plant seen in South India. It is mentioned in Ayurvedic classics by the name *Rajapatha*and is one of the important drugs used in Ayurveda therapeutics and is used widely in different formulations in medical practice. The quality control of herbal crude drugs and their bio constituents is of paramount importance in justifying their acceptability. The crude drugs can be identified systematically on the basis of their morphological, histological, chemical, physical and biological studies. Phytochemical screening reported the presence of alkaloids, carbohydrates, glycosides, phyto sterols compounds and proteins and amino acids. The plant widely used in the treatment of cough, fever, kidney disorder, urinary disorder and snake poisoning.Aim of the present study is to extraction and isolation of bioactive compound (Thymol) from *Cycleapeltata*(Lam) Hook. f. Thoms. The isolation and characterization analysis included Thin Layer Chromatography, High Performance Thin Layer Chromatography, GC-MS and Spectroscopy studies (IR, NMR, and MASS).The presences of thymol in the ethanolic fraction revealed that the isolated constituent is most active compound. Isolation of bioactive compound thymol may help in identification of various pharmacological activities and carrying out further research in *C.peltata*.

**Keywords:** Menispermaceae, *Cycleapeltata,* thymol,GC-MS, Isolation,Characterization

## Introduction

*Cycleapeltata*(Lam.) Hook.f. and Thomson is a member of the family Menispermaceae, which is also known as Patha root in English. In ayurveda, it is well known as rajapatha, and is mentioned in most of the ayurvedic texts like Charaka Samhita, Sushrutasamhita and Ashtangahridya.[1] *Cycleapeltata*is a much-branched, climbing shrub found throughout South and East India.[2] Roots are tuberous, leaves peltate, hairy 2- 5 cm long, flowers are very small. Male flowers in panicles, very long. Fruit-drupe, reniform. The species of this plant are easily distinguished by the cup-shaped calyx and corolla.[3]

**Fig.1 [4]Cycleapeltata**

| **Scientific Classification** | |
|---|---|
| Kingdom: | Plantae |
| *Clade*: | Tracheophytes |
| *Clade*: | Angiosperms |
| *Clade*: | Eudicots |
| Order: | Ranunculales |
| Family: | Menispermaceae |
| Genus: | *Cycle*[5] |

*Cycleapeltata*is widely used in the treatment of cough, fever, kidney disorder, urinary disorder and snake poisoning. Powdered roots are used for the treatment of diabetes, tooth ache, decoction of the roots and leaves are used for treating malaria and asthma.[6]The plant contains various chemical constituents of alkaloids, carbohydrates, glycosides, phyto sterols compounds and proteins and amino acids. Other alkaloids such as bisbenzylisoquinoline alkaloids, tetrandrine, tropoloisoquinoline alkaloids such as pareirubrine A and B, etc., are reported which contributes to its pharmacological properties. [7]

## Materials and Methods

### Collection of Plant Material

The fresh sample leaves of *Patha*(*Cycleapeltata*(Lam) Hook. f. Thoms) were collected from their natural habitat near Sasthamcotta, Kollam district, Kerala.[8] Samples were authenticated(PHARM 17 BO3) by the pharmacognosist and the voucher specimens were kept in Lab of Dept. of Aromatic and medicinal plants research station, Kerala agricultural University, Kerala. They were shade dried and packed in zip lock polythene bag and labeled.


**Fig.2 *Cycleapeleata*Plant Extract**

### Extraction

The collected plant materials were washed twice in running tap water and shade dried at room temperature for 3 weeks. The air-dried plant leaves were pulverized, using an electric blender to make a fine powder. A total of 3 kg of powdered *C. peleata*leaves was sequentially extracted with ethanol using a Soxhlet apparatus until the efflux solvents become colorless. The after Extraction the extract passed through the Whatman filter Paper (Whatman No. 1) to avoid impurities and dried under vacuum at 40◦C. The dried crude methanol extract was stored in a freezer at −4◦C for further study [9].

### Separation of Active Compounds by Chromatography (Thin Layer Chromatography)

**Fig.3 Column Packing**

The column is to be prepared by plugging the lower part of the column with small amount of pre-extracted quartz wool (or glass wool), and by running with non-polar solvent mixture. This step is to be followed by addition of activated silica gel (or alumina) to the column to the required volume. Finally, extractable organic matter of the sample to be fractionated has to placed on top of the column. Plugging the lower part of the column with small amount ofor glass wool), and by rinsing with polar solvent mixture. This step is to be followed by addition of activated silica gel (or alumina) to the column to the required volume. Finally, extractable organic matter of the sample to be fractionated has to be placed on top of the column.

The residue was chromatographed on silica gel preparative slides using different solvent systems: acetone, hexane and ethyl acetate, separately, or combined solvent systems; acetone: ethyl acetate (1:2), acetone: ethyl acetate. (2:1), hexane: ethyl acetate (1:2), and hexane: ethyl acetate (2:1). The starting crude spots were observed for migration and separation by the previously prepared mobile phases. R values of the obtaining colored and non-colored spots with the aid of visible and UV lamps were recorded. Using silica gel plates (20· 20 cm dimensions and 0.50 mm thickness of 60GF254 fine grade), the active bands were gathered, dissolved in ethyl acetate and concentrated to dryness in the vacuum. The dried TLC plate was viewed by iodine vapor and visualized under UV light (low and high wavelength). [10]

The culture broth (10 L) was extracted with ethyl acetate (1:1v/v) stepwise and concentrated by rotary evaporator at 50°C to yield 2 g of brown crude residue. The residue was chromatographed on silica gel preparative slides using different solvent systems: acetone, hexane and ethyl acetate, separately, or combined solvent systems; acetone: ethyl acetate (1:2), acetone: ethyl acetate. (2:1), hexane: ethyl acetate (1:2), and hexane: ethyl acetate (2:1). The starting crude spots were observed for migration and separation by the previously prepared mobile phases. R values of the obtaining colored and non-colored spots with the aid of visible and UV lamps were recorded. Using silica gel plates (20· 20 cm dimensions and 0.50 mm thickness of 60GF254 fine grade), the active bands were gathered, dissolved in ethyl acetate and concentrated to dryness in the vacuum.[11]The ethanol extract of each fraction was purifiedand analyzed by GC–MS and HPLC. The structure of isolated compound was characterized by spectral studies UV, FT-IR, NMR and MASS studies.

**Gas chromatography–Mass Spectrum[12, 14]**

The active fraction was analyzed using the SHIMADZU GC–MS-QP5050A with programm CLASS 5000 in the Central Lab facility. Identification was performed using WILEY MASS SPECTRAL DATA BASE Library.

**Infrared[13]**

One mg sample of extracted crude was subjected to IR-spectral analyses using Infrared Spectrophotometer. Mid IR region of 400–4000 cm-1 was used for sample analysis. A mixture of spectroscopic pure KBr was in the ratio of 5:95; pellets were fixed in sample holder.

**Result and Discussion**

**Thin Layer Chromatography Separation**

Separation of the compounds standards by TLC by ethanol extract of *C. pelata* was dissolved in 50 mL of hot water and extracted three times with 50 mL of ethyl ether and ethyl acetate, respectively. Solvents from all of the fractions were removed with a rotary evaporator to obtain the ethyl ether and ethyl acetate extract. The ethyl ether extract was separated byTLC (normal-phase plates, ethyl ether–hexane, 1:5, v/v), and the nine zones found on the TLC plate could be visualized underUV light at 254 nm. Each zone was scraped from the plate and extracted with ethanol. The ethanol extract of each fraction was analyzed by GC–MS and HPLC.

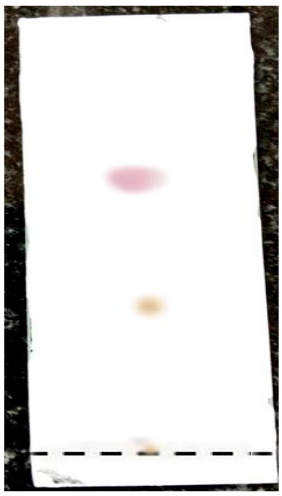

**Fig.4TLC of Thymol**

Based on the Rf values of the bands the active principles were identified with standard. Under UV light – for terpenoid system.TLC of thyme visualized with UV light) shows spots, and based on relative Rf values, After elution, the purity of each fraction was tested by analytical TLC which showed clear separation of fractions. This fraction was scraped and collected (Fig.4) for further analysis.

**GC-MS Analysis**

In GC –MS Analysis there are many compound identified and named as **(519- Penene, 566 – Isoquinolinamine, 602- benzene, 650- Thymol, 720- Eugenol and 778- Dodecane,** GC –MS spectra given in Fig.5. Among them further the Thymol is focused and subjected to isolation ,purification procedure and the structure of isolated compound Thymol was identified by spectral studies.[15]

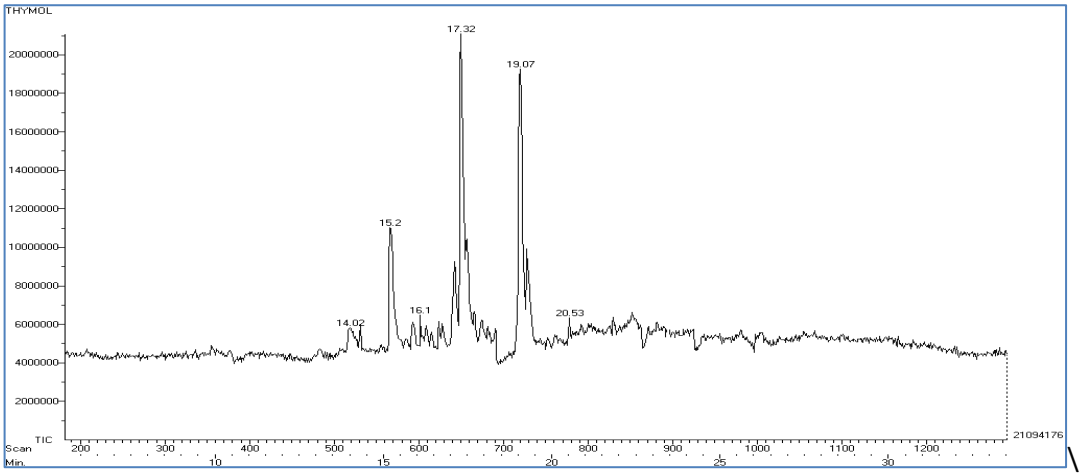


**Fig.5 GC-MS- Analysis of *Cycleapeleata***

**HPLC Analysis**

The purity of isolated compounds was checked by HPLC analysis and spectra recorded and given in Fig.6. The isolated active compound shows a separation peak at a retention time of 27.1min for active compounds which is compared with standard respectively. The purity of the active compounds (Thymol) was indicated as a single sharp peak.

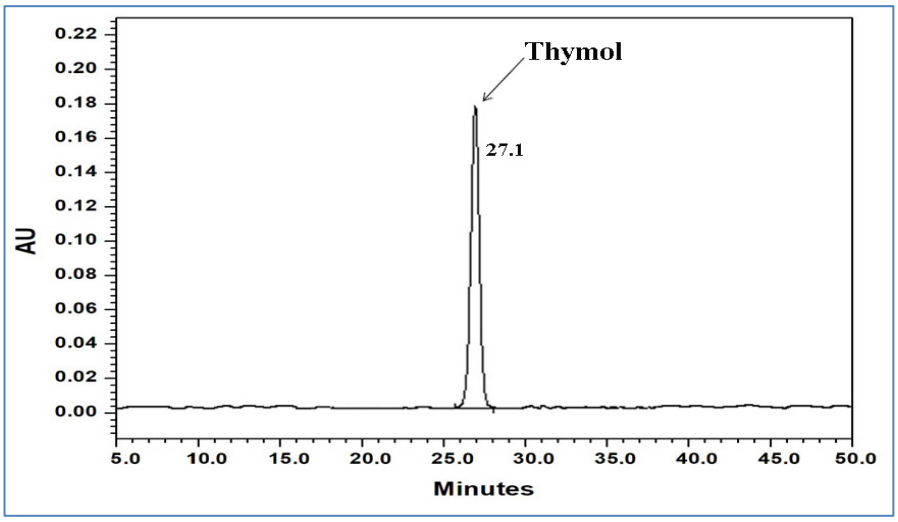


**Fig.6 HPLC (Thymol Separation)**

**UV- Spectrum Analysis of Isolated Compound**

As per the reference the UV visspecFig.7) analysis the isolated fraction of Thymol shows 276 nm peak, confirmed as Thymol with the reference. [16]

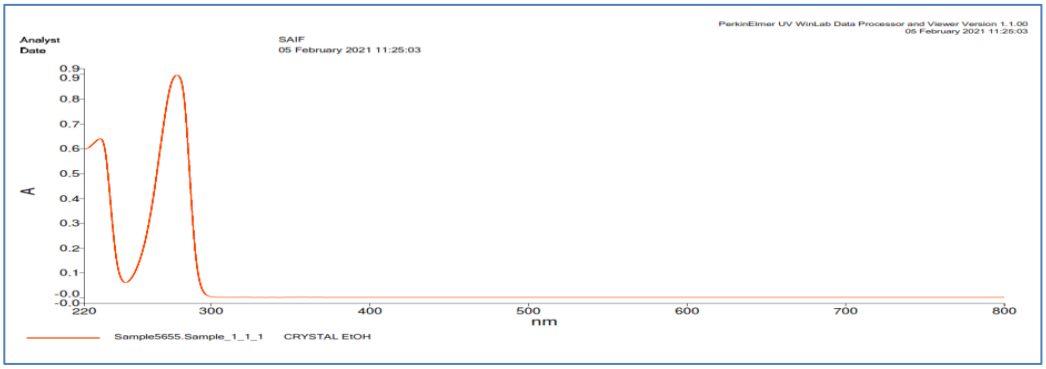


**Fig.7 UV-Vis Spec of Thymol**

**FTIR- Spectrum Analysis of Isolated Compound**

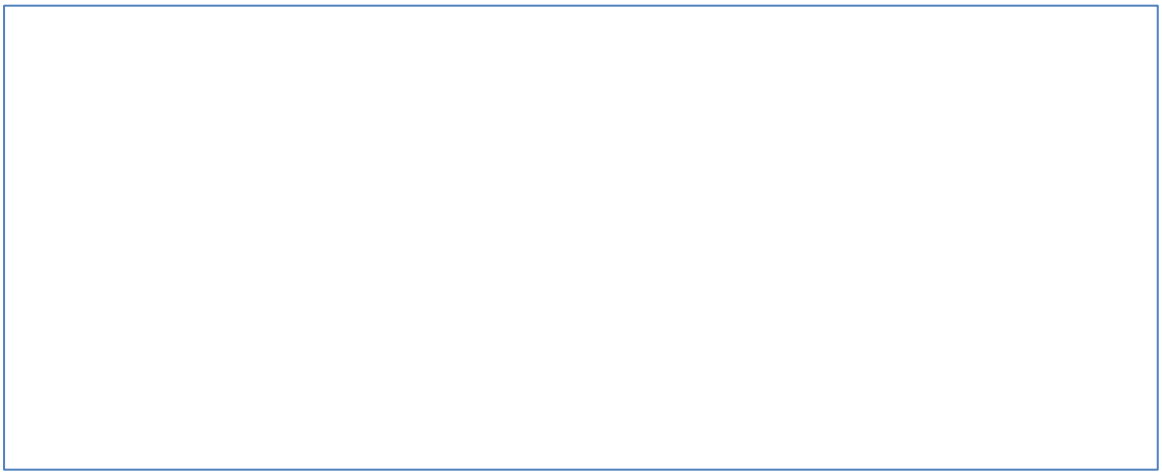

**Fig.8 FTIR- Spectrum of Thymol**

FTIR spectrum(Fig.8) of purified Thymol shows many peaks corresponding to functional groups which are present in structure of isolated compound there were a broad peak at 3173.71 cm−1, which corresponds to the Hydroxyl group (O-H). The aromatic C–H stretching peak was observed at 3031.78 cm−1 while aliphatic C-H stretching was observed in 2886.89cm−1,C=C stretching frequency was found at 1618.30cm−1. The absorption band at 1873.55 and 1156.81 cm−1 corresponds to the C-C stretch of Benzene ring and C-O bend, respectively.[16]

**NMR- Spectrum Analysis of Isolated Compound**

NMR of Thymol and it was found that the coupling constants JAA' and JBB' change whereas the constants JAB and JAB' remain practically unchanged. The chemical structure of thymol from the NMR

peak assignments of proton and carbon signals were in accordance with the reference literature. The spectrum (Fig.10) shows two sets of two chemically equivalent aromatic protons, predicted from the integration values, resonating at δ 7.32 and 6.89 ppm. The two protons, close to the inductive electron withdrawing effect of the oxygen atom were assigned to signal at δ 7.32 (2H, d, J = 8.8,). The other two aromatic protons were assigned to signal at δ 6.89 (2H, d, J = 8.8,).The structure of Thymol was detected as a singlet peak at δ 6.44 which is respective to hydroxyl potons. It is well separated from signals of the other compounds. The isolated compound Thymol, typical Phenolic structure signals NMR analysis of the isolated fraction compared to existing literature.[16]

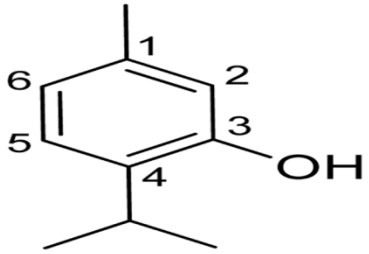


**Fig.9 Thymol Structure**

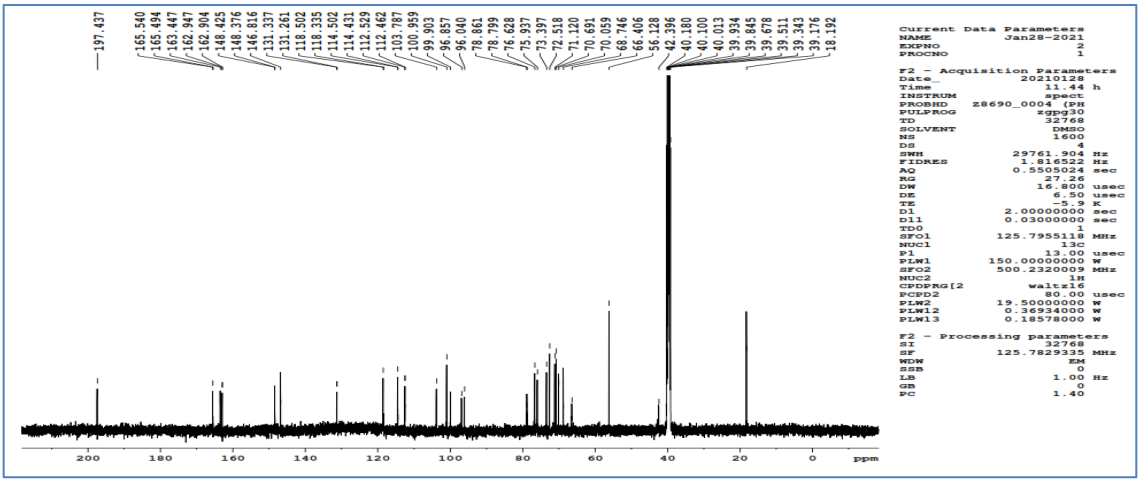


**Fig.10 NMR Spectrum of Isolated Thymol**

**Mass of Thymol**

Through the Mass spectrometric analysis the isolated compound identified as Thymol with the molecular weight of 150.22. Mass spectral analysis suggested MS at m = z 134 of compound. It was represented in this data that this compound corresponds to a molecular formula of $C_{10}H_{14}O$.

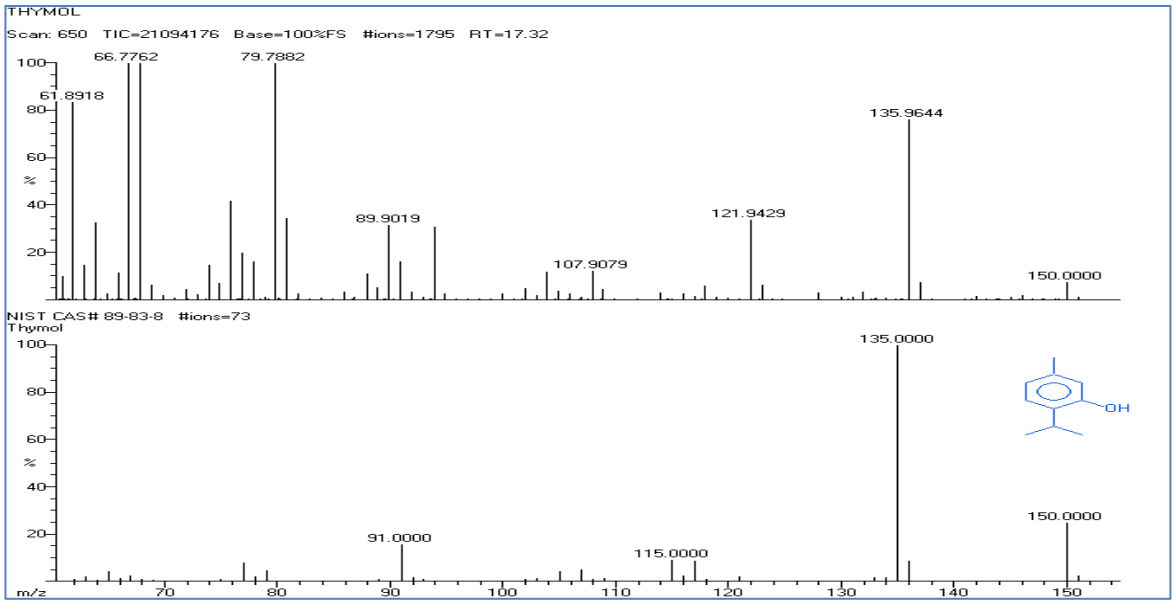


**Fig.11 Mass Spectrumof Thymol**

## Conclusion

The bioactive compound was isolated by chromatographic techniques (TLC), Purified using HPLC and GC-MS analysis. Finally the structure of the compound was appraised by FTIR, NMR and MASS spectroscopy studies which revealed that the isolated compound is Thymol compare with the standard. From the above result conclude that the ethanolic extract of plant *C.Peltata* is good source of thymol. Hence, this study recommends that the isolated active compound thymol can be used as a prototype molecule for medicinal drug.

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