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ISOLATION, CHARACTERIZATION AND PHYTOCHEMICAL EVALUATION OF ACTIVE COMPOUND THYMOL FROM *CYCLEA PELTATA* LAM

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Keywords:

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ABSTRACT: *Cyclea peltata* (Lam), belonging to the family *Menispermaceae,* is a common plant in South India. It is mentioned in Ayurvedic classics by 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 crude herbal drugs and their bio constituents is paramount 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 is widely used in the treatment of cough, fever, kidney disorder, urinary disorder, and snake poisoning. The aim of the present study is to extract and isolation of bioactive compound (Thymol) from *Cyclea peltate* (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 presence of thymol in the ethanolic fraction revealed that the isolated constituent is the most active compound. Isolation of bioactive compound thymol may help in the identification of various pharmacological activities and carrying out further research in *C. peltata*.

INTRODUCTION: *Cyclea peltata (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, Sushruta Samhita and Ashtanga hridya [1]. *Cyclea peltata* 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].

Cycleapeltatais widely used in the treatment of cough, fever, kidney disorder, urinary disorder and snake poisoning. Powdered roots are used for the

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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, phytosterols compounds, proteins and amino acids. Other alkaloids such as bisbenzylisoquinoline alkaloids, tetrandrine, tropoloisoquinoline alkaloids such as pareirubrine A and B, *etc.*, are reported, contributing to its pharmacological properties [7].

FIG. 1: *CYCLEAPELTATA*[4]

Scientific Classification:

Kingdom	:	Plantae
Clade	:	Tracheophytes
Clade	:	Tracheophytes
Clade	:	Eudicots
Order	:	Ranunculales
Family	:	Menispermaceae
Genus	:	*Cycle*[5]

MATERIALS AND METHODS:

Collection of Plant Material: The fresh sample leaves of Patha *Cyclea peltata* (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 the Lab of Dept. of Aromatic and medicinal plants Research station Odakkali Kerala agricultural university. They were shade dried and packed in a zip lock polythene bag and labeled.

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].

FIG. 2: *CYCLEA PELEATA* PLANT EXTRACT

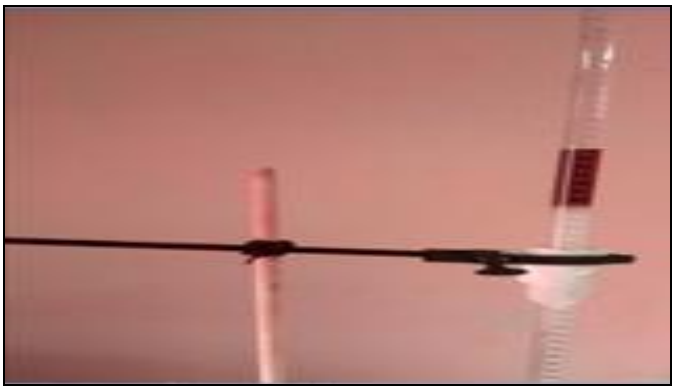

FIG. 3: COLUMN PACKING

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 place on top of the column. Plugging the lower part of the column with small amount for 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 chromate graphed 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 a rotary evaporator at 50 °C to yield 2 g of brown crude residue. The residue was chromate graphed 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 purified and analyzed by GC-MS and HPLC. The structure of isolated compounds 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 program CLASS 5000 in the Central Lab facility. Identification was performed using Wiley mass spectral database 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 the 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 by TLC (normal-phase plates, ethyl ether–hexane, 1:5, v/v), and the nine zones found on the TLC plate could be visualized under UV 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. 4: TLC OF THYMOL

Based on the R_f values of the bands, the active principles were identified with the standard. Under UV light - for terpenoid system. TLC of thyme visualized with UV light) shows spots and based on relative R_f 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 compounds 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 isolated compound Thymol's structure was identified by spectral studies [15].

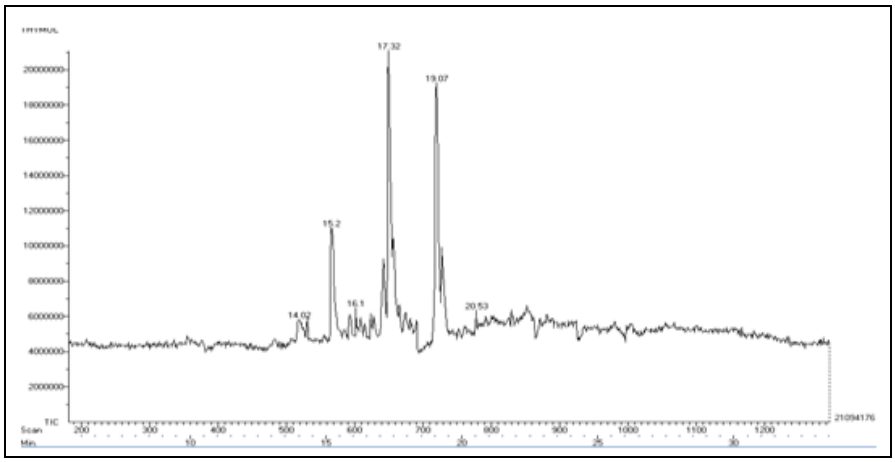

FIG. 5: GC-MS- ANALYSIS OF *CYCLEAPELEATA*

HPLC Analysis: The purity of isolated compounds was checked by HPLC analysis and spectra were recorded and given in **Fig. 6.**

The isolated active compound shows a separation peak at a retention time of 27.1 min for active compounds compared with standard respectively. The purity of the active compounds (Thymol) was indicated as a single sharp peak.

UV- Spectrum Analysis of Isolated Compound: AS per the reference the UV visspec **Fig. 7.** analysis the isolated fraction of thymol shows 276 nm peak, confirmed as Thymol with the reference.

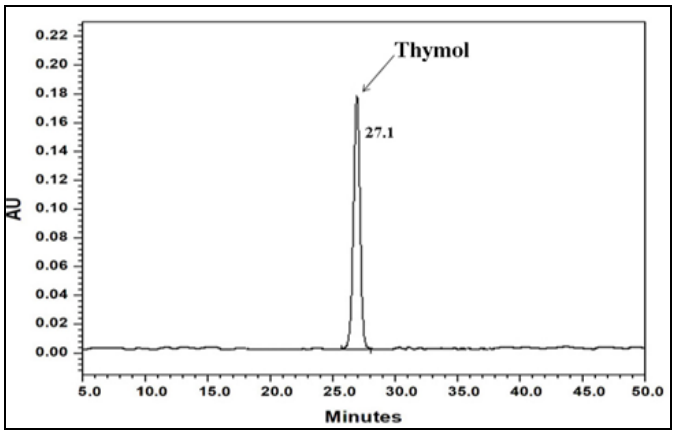

FIG. 6: HPLC (THYMOL SEPARATION)

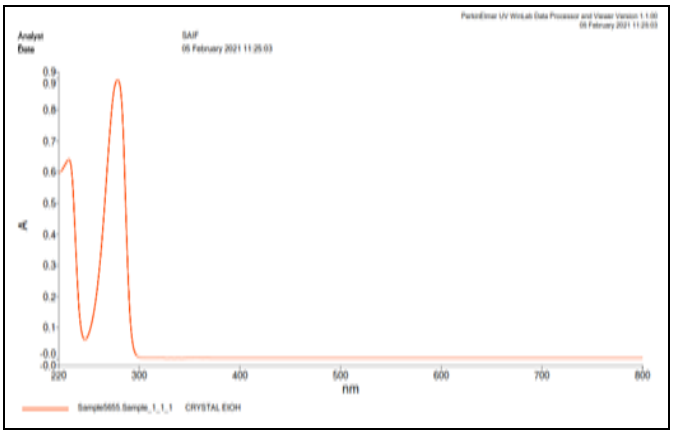

FIG. 7: UV-VIS SPEC OF THYMOL

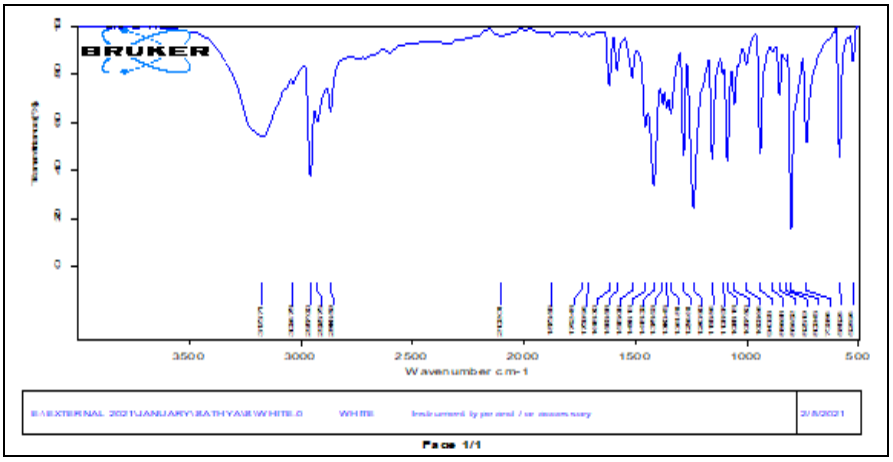

FIG. 8: FTIR- SPECTRUM OF THYMOL

FTIR- Spectrum Analysis of Isolated Compound: FTIR spectrum **Fig. 8.** of purified Thymol shows many peaks corresponding to functional groups present in the structure of the isolated compound. There was 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 2886.89 cm^{-1},C=C stretching frequency was found at 1618.30 cm^{-1}. The absorption band at 1873.55 and 1156.81 cm^{-1} corresponds to the C-C stretch of the 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 was 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 single peak at δ 6.44, which is respective to hydroxyl protons. It is well separated from the signals of the other compounds. The isolated compound thymol, typical Phenolic structure signals NMR analysis of the isolated fraction compared to existing literature [16].

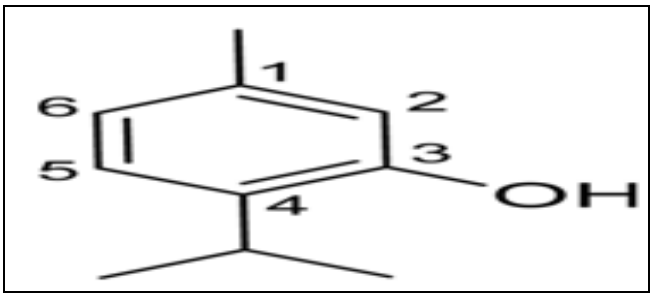

FIG. 9: THYMOL STRUCTURE

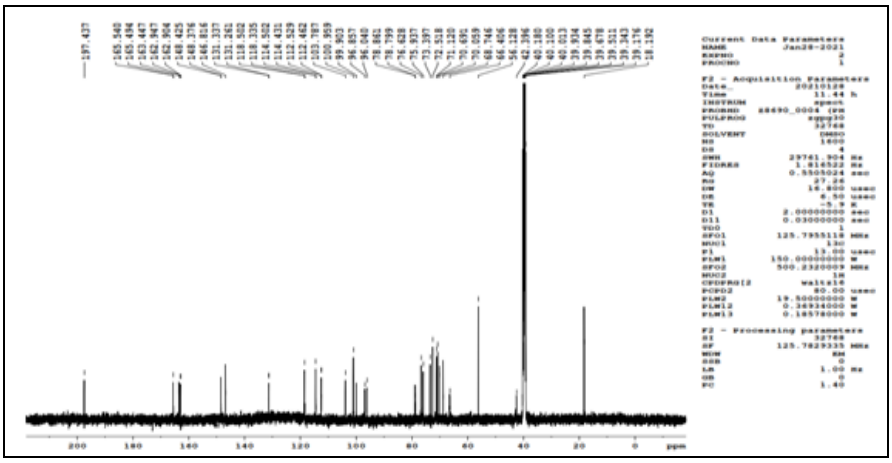

FIG. 10: NMR SPECTRUM OF ISOLATED THYMOL

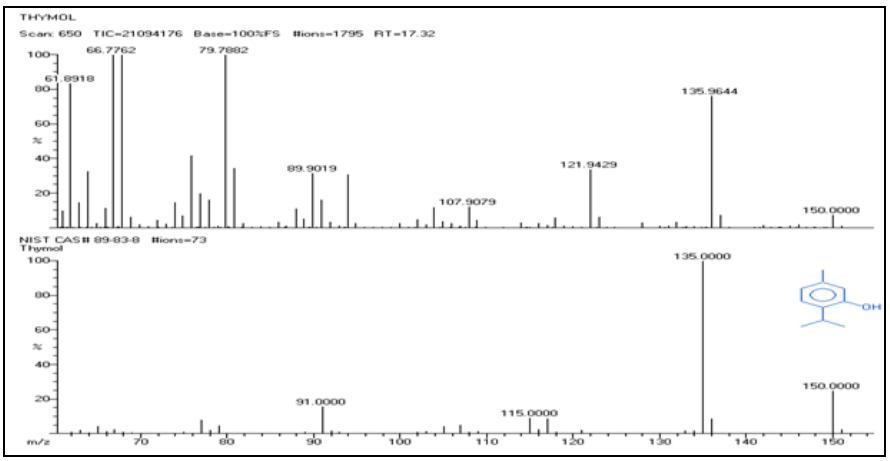

FIG. 11: MASS SPECTRUMOF THYMOL

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. The above result concludes that the ethanolic extract of plant *C. peltata* is a good source of thymol. Hence, this study recommends that the isolated active compound thymol can be used as a prototype molecule for the medicinal drug.

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CONFLICTS OF INTEREST: The authors declare no conflicts of interest, financial or otherwise.

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