BIO ASSAY GUIDED FRACTIONATION AND STRUCTURAL DETERMINATION OF ISOLATED COMPOUNDS OF *ERYCIBE PANICULATA ROXB.*

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ABSTRACT:

Pharmacological screening of plant preparations, followed by bioassay guided fractionation that isolates pure active plant ingredients, is one of the effective approaches for exploring traditional medicines as a source of novel medications. Hence, the aim of present study is to determine bioactive compound from LPE extracts of *Erycibe paniculata Roxb*. Elution of LPE extracts is done using column chromatography. We have gathered eight fractions designated from LPE-1 to LPE-8 and are tested against different bacterial strains. Out of all LPE-4 shown notable efficacy against tested bacteria. TLC is carried out for LPE-4 and the structural determination of active compound is done using spectral analysis (1HNMR). ^{1}HNMR spectral analysis of LPE-4 fraction was identified as 2-3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one.

1.0 INTRODUCTION

The antibacterial activity and phytochemical analysis of leaf extracts from *Erycibe paniculata Roxb*. discovered earlier were considered to be important in inhibiting some bacterial strains **(**Supriya and Krishna, 2024**)**. Therefore, Leaf Petroleum Extract (LPE) was chosen from among the leaf crude extracts to undertake bioassay guided fractionation in order to extract the bioactive components from LPE. One important technique for separating chemicals from plant extracts is bioassay-guided fractionation (Marupati et al., 2019).

Tests were conducted against certain bacterial species utilizing the fractions that were separated using column chromatography (100-200 mesh). To determine how many compounds are

present, the active fractions will be marked on the TLC sheet. Spectral analysis will be used to determine the structure of the single spot and active TLC plates.

According to Swarnendu Mondal and Chowdhury (2020), after careful consideration of the literature, the stem bark of *E. paniculata* contains a variety of secondary metabolites, including alkaloids, flavanoids, steroids, triterpenoids, glycosides, cardic glycosides, gums, tannins, saponins, anthraquinones, amino acids, lignins, and proteins. The authors used GC-MS to identify nine chemicals. Two of the nine are fatty acid esters (Cyclopropanedodecanoic acid, 2-octyl-, methyl ester (A) and 10-Heptadecen-8-ynoic acid, methyl ester, (E)-) (B); three are carboxylic acid esters (Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (C); 1,2-Benzenedicarboxylic acid, butyl octylester (D); 2-Propenoic acid, 3-(3,4,5-trimethoxyphenyl)-, methyl ester) (E); three are coumarin derivatives (benzopyrone family member with lactone-like chain), (2-Hexadecanol (F); scoparone (G); and the final one is the aromatic ketone (ether) 2,4,6-Trimethoxyacetophenone.

2.0 MATERIALS AND METHODS

Based on the therapeutic properties of leaf petroleum extract (LPE) of *E.paniculata,* the present paper deals with the bioassay-guided fractionation of LPE to isolate the responsible compound for the activity.

2.1 ELUTION OF E. *PANICULATA* LEAF PETROLEUM ETHER EXTRACT (LPE)

About 100–200 mesh of silica gel was used to pack the column chromatography (slurry technique) (Praveen Kumar and Thupurani Murali Krishna, 2023). To establish the column bed, petroleum ether was poured into the column and it was operated for 15 to 20 minutes. We moved the crude leaf extract made with petroleum ether (LPE) to the top of the column. The chemicals in the extract were eluted using a dual mobile phase (petroleum ether: toluene). All eight of the fractions we have gathered are designated LPE-1 to LPE-8. LPE-4 shown notable efficacy against tested bacterial strains among LPE-1 to LPE-8; however, the findings are simply displayed as active or not active (Table 1.1), and no information pertaining to the zone of inhibition is displayed here. The TLC pattern was applied to the active fraction (LPE-4). There was only one place on the sheet as a consequence. Moreover, 1HNMR spectra were used to clarify the compound's structure.

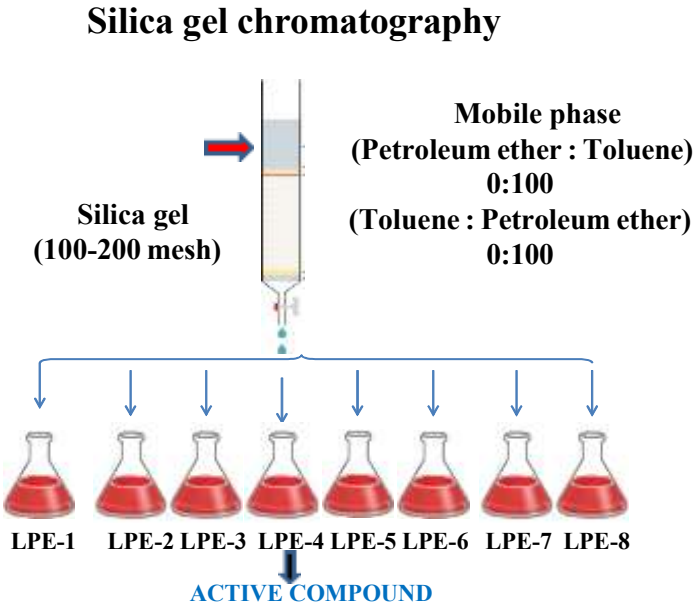
Table 1.1 represents the antibacterial activity of column fractions separated from the LPE

Fraction	MRSA	*B. subtilis*	*B. cereus*	*P.aeruginosa*	*E.coli*	*S. typhi*
LPE-1	NA	NA	NA	NA	NA	NA
LPE-2	NA	NA	NA	NA	NA	NA
LPE-3	NA	NA	NA	NA	NA	NA
LPE-4	ACTIVE	ACTIVE	ACTIVE	ACTIVE	ACTIVE	ACTIVE
LPE-5	NA	NA	NA	NA	NA	NA
LPE-6	NA	NA	NA	NA	NA	NA
LPE-7	NA	NA	NA	NA	NA	NA
LPE-8	NA	NA	NA	NA	NA	NA

(LPE: Leaf Petroleum Ether; NA: Not-Active)

3.0 RESULTS

According to the ^{1}HNMR spectra the LPE-4 fraction was identified as **2-3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one**.



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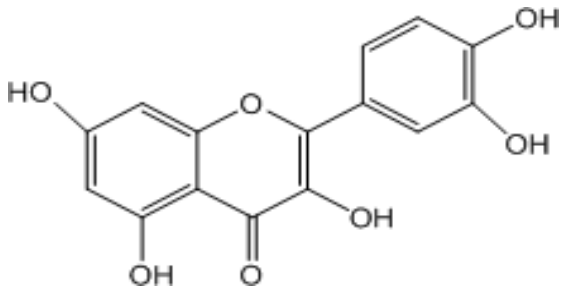
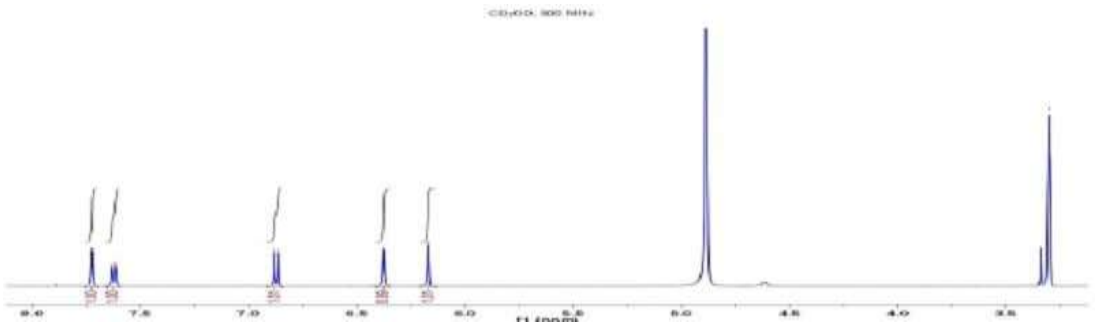


2-(3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one

Fig 1.1 Column chromatography fractions and isolated compound from E. *paniculata* leaf petroleum ether crude extract



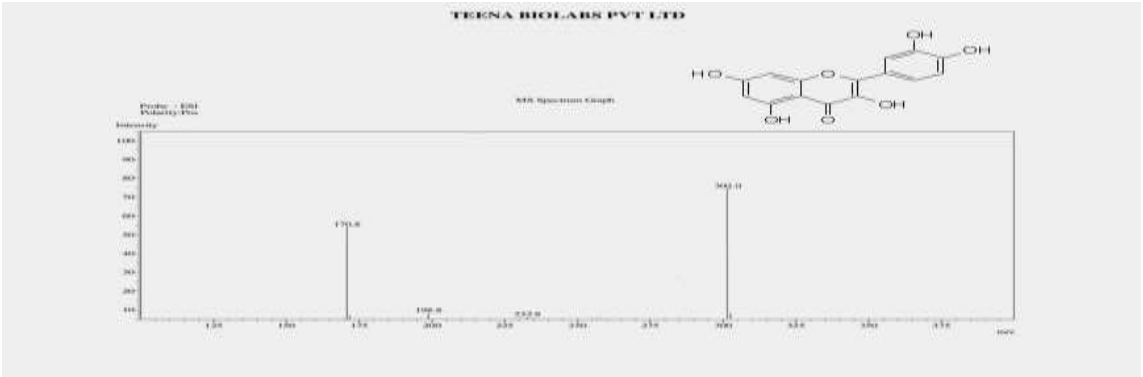


Fig 1.2 [1]HNMR and Mass spectra of the 2-3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one

Spectral Data

White needles. MS: 286. 1H NMR (200 MHz, DMSO-d6): d: 10.92 (OH), 9.41 (OH), 9.35 (OH),9.25(OH), 7.92 (1H, d, J = 9.4 Hz, H-5), 7.45 (1H, d, J = 2.0 Hz, H-20), 7.78 (1H, s, J = 8.6 and 2.2 Hz, H-60), 7.62 (1H, s, H-20 , H-50 , H-6),7.81(1H,s,. 13C NMR (50 MHz, DMSO-d6): d: 177.28 (C-4), 171.14 (C-7), 162.36 (C-9), 155.23 (C-40), 151.23 (C-30), 148.23 (C-3), 127.51 (C-10), 121.45 (C-5), 120.41 (C-60), 119.63 (C-50), 116.45 (C-20), 115.72 (C-10), 1.27 (C-6), 113.25 (C-8).

Compound Name	Quercetin hydrate
IUPAC Name	**2-3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one**
Molecular Formula	$C_{15}H_{12}O_8$

4.0 DISCUSSION

The development of new and innovative medications from natural sources such as microbes and plants has gained significant attention worldwide (Newman and Cragg, 2020). It has been observed that the majority of modern chemically manufactured medications have harmful effects on people (Gunderson and Haughey2014; Schifano et al., 2015; Tait *et al.,* 2016).

On the other hand, the fast emergence of multidrug-resistant pathogenic bacteria or cancer cells possesses a significant risk to clinical settings and makes treating various bacterial illnesses and malignancies more challenging. Thus, the development of medications with strong therapeutic benefits and no adverse effects has been taken into consideration globally.

With reference to the drug discovery from different sources, we have isolated one compound **(2-3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one)** from the Leaf Petroleum ether extract (LPE) using bioassay guided fractionation. Based on structure elucidation, we have identified this compound as quercetin hydrate.

Swarnendu Mondal and Chowdhury (2020) identified fatty acid esters, carboxylic acid esters, coumarin derivatives, and aromatic ketone, according to the review. Using GC-MS, nine components (A-I) in all were separated from the methanolic extract of E. paniculata. Cyclopropanedodecanoic acid, 2-octyl-, methyl ester (A), 10-Heptadecen-8-ynoic acid, methyl ester, (E)-) (B) (fatty acid esters (A-B)) and Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (C); 1,2-Benzenedicarboxylic acid, butyl octylester

(D); 2-Propenoic acid, 3-(3,4,5-trimethoxyphenyl)-, methyl ester) (E) (carboxylic acid esters (C-E) and (benzopyrone family member with lactone-like chain); scoparone (G); scopaletin (H) (coumarin derivatives (F-H)); and 2,4,6-Trimethoxyacetophenone (I) (aromatic ketone (I) are the isolated compounds. Quercetin hydrate, also known as 2-3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one, has been isolated from a variety of plants (Havsteen,.2002**;** Mali and Gawande, 2019). However, for the first time we have isolated this compound form *E. paniculata* leaf petroleum ether crude extract.

5.0 CONCLUSION

Based on the findings of the bioassay-guided fractionation, the active component that gives the activity for leaf petroleum crude extract has been identified in the column fractions. We also concentrate on the extracted compound's antibacterial effectiveness against the previously purchased microbial strains in order to verify its efficacy.

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