The Chemist
Journal of the American Institute of Chemists

Structural Elucidation of Ethanol Extraction of *Plectranthus zeylanicus*

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Abstract: *Plectranthus zeylanicus,* is an aromatic herb in the family of Lamiaceae. It is widely used in Sri Lanka for making herbal medicines. *Plectranthus zeylanicus* is often called *Coleus zeylanicus*, and is locally known as *iruveli* in India. It is used in Aayurvedic medicines and effective in the treatment of asthma, cough, vomiting, diarrhea, throat infection, dysentery, fever, chronic ulcers, dental and eye diseases. This plant contains enzymes and flavonoids. The leaf and stem of the plant were subjected to solvent extraction and the ethyl acetate fraction is purified for obtaining a single compound. The compound was characterized by UV Visible, FT-IR, ^{1}H NMR, and LC-MS studies and investigated for its possible structure.

Key Words: *Plectranthus zeylanicus,* UV Visible, ^{1}H NMR , FT-IR and LC-MS

1. Introduction

Plant based medicines (Aayurveda) are safer alternatives to many synthetic medicines. They have fewer side effects, and the body can absorb these bio-compounds easily [1-4]. Even though this natural medicine is considered to have many advantages, the main drawback is that it needs more curing time, and larger amounts of the plants are essential for the drug's preparation [5-9]. The main alternate solution for such problems is to concentrate the particular components of the plant which cures and to synthesize the same chemically [10-11]. So, we may have to consider the whole plant extract and it's chemical components, which are actually responsible for healing [12]. While choosing the synthesis of the same chemically, we can minimize its excess use and presence of other components in the plant which may cause complications [13]. That is, we may suggest the medicinal compound in pure form through chemical

synthesis without destroying the natural sources.

Plectranthus zeylanicus (PZ) is a perennial herb, cultivated in Sri Lanka and in India. This plant is known as *karpuravalli* and *iruveli* in Malayalam and *kannikkaurkk*a in Sanskrit. Because of the presence of bioactive secondary metabolites, *PZ* is used in many traditional drugs [14-16]. The plants as a whole or as various extracts are used for the treatment of dysentery, eye disorders, vomiting, dermatitis, throat infection, cough, fever, and ulcers. The major component of this plant is α-terpeneol [17]. More than 100 compounds of this plant were detected, presented in small amounts. That is, only 60% of the components were identified. P-cymene, geraniol and geranyl acetate were found. 6β, 7β- dihydroxy-royleanone were also identified. It contains aromatic chemical compounds and essential oils, and has anti-inflammatory, antifungal, antimicrobial and disinfectant activities. These plants are very important for the perfume and medical industry [18].

Mayuri Napagoda et al. [19] have isolated dichloromethane extract of PZ and obtained the compound 7α-acetoxy-6β-hydroxy-royleanone. In another study, Mayuri Napagoda et al. [21] revealed 5 lipoxygenase(5-Lo) inhibition of PZ, which has an important role in preventing disorders like asthma or atherosclerosis. They have studied the anti-inflammatory activity of the isolated phytoconstituent *in-vitro*. They also studied its antibacterial and antifungal activities and claimed this as a remedy for inflammatory disorders and microbial infections.

Sudhara G. De Soyaza et al. [20] studied the antimicrobial activity of silver nanoparticle prepared from *PZ*. They have studied the hexane extract of this plant and indicate the potent inhibition of 5-lipoxygenase enzyme. They have also claimed the presence of diterpenoids, pentacyclic triterpenes, fatty acids, phytosterols, etc., in the extract.

In this paper, we describe the extraction of *PZ* using ethanol solution. The green plants planted in our farm were collected, washed and dried in shade [21-23]. The plant was identified by a botanical expert. They were subjected to polarity index extractions, using organic solvents. For the specific study, we collected the ethanol extract. Our aim was to extract the phytochemical components, separate and purify and reproduce them in high concentration and use them in the affected areas [24-26].

2. Experimental

Materials

Plectranthus zeylancius (Figure 1), Hexane, Ethyl Acetate (E Merck, India), Ethanol (commercial grade). All solvents were distilled before use.

Fig 1. Image of *Plectranthus zeylancius*

Instruments

UV spectra were recorded on an Ultraviolet-visible spectrophotometer (Jasco V-650), FT-IR spectrum was produced by Fourier transform infrared spectrometer (FT-IR) (Jasco model 4100) using KBr pellets. Liquid Chromatography Mass Spectrum was obtained by Agilent 6100 Series Quadrupole. The NMR spectral study was carried out at 400 (^{1}H) and 100 (^{13}C) MHz on a Bruker-400 MHz spectrometer instruments. The chemical shift values are reported relative to Me_4Si (^{1}H) and $CDCl_3$ (^{13}C) as internal standards. The value of coupling constant (J) was stated in Hertz (Hz).

Procedure

The dried and powdered sample was used for the analysis. It was extracted using solvents of increasing polarity. First, hexane was used. The components soluble in hexane were separated out. The second extraction was carried out with ethyl acetate and the compounds were separated. In the third step, we selected ethanol as a solvent. 30 g of the powder of stem and leaf of *PZ* was transferred to the round bottom flask and extracted using ethanol as a solvent for a time span of three hours. It was filtered and the ethanol extract was collected. The solvent was removed. The dry army green coloured compound, thus obtained, was subjected to TLC and then purified by column chromatography. A single component was obtained (Figure 2) and used for further study.

Fig 2. Image of the Extracted Compound

The structural elucidation was carried out using UV, IR, NMRs and LC-MS spectra and information from literature.

3. Results and Discussion

As a whole, we have obtained a very low yield of 800 mg pure compound from 30 g of starting material. For the structure elucidation of the compound, the following spectra were recorded.

Extract	Colour	Yield	Melting point (°C)	Nature
Ethanol extract	Army Green	800mg	> 360	Powder

Spectral Details

Figure 3 shows the UV-visible spectrum of ethanol extract of *PZ.* It shows three peaks at 286, 310 and 664 nm. The first peak (286 nm) indicates the presence of unsaturation (π-π* transitions) and the second peak (310 nm) for a carbonyl group (n- π*transitions). The peak obtained in the visible region (664 nm) is of its colour.

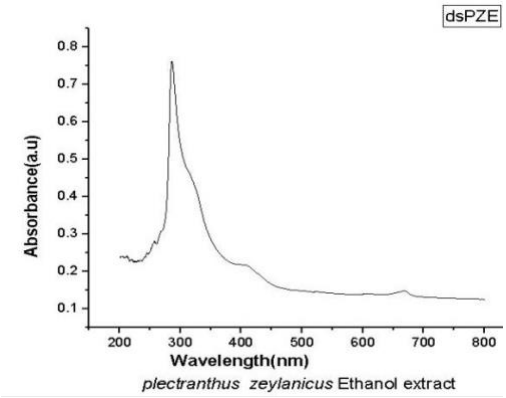


Fig 3. UV-Visible Spectrum of Ethanol Extract of *PZ*

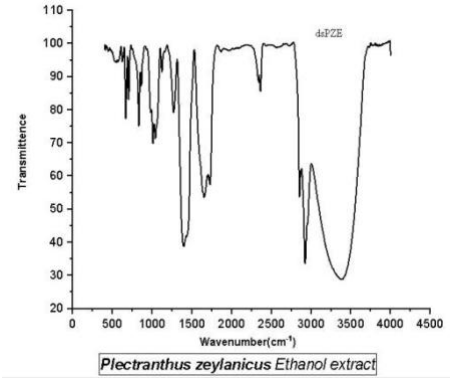


Fig 4. IR Spectrum of Ethanol Extract of *PZ*

Figure 4 shows the IR spectrum of ethanol extract of *PZ*. The peaks (cm^{-1}) obtained are at 3557 (OH), 3345 (C-H str of C=C), 2918 (C-H str of CH_3), 2849 (C-H str of CH), 1716 (C=O), 1644 (C=C bend), 1603 (C-N), 1383 (C-H bend), 1262 (C-O aromatic), 1116(C-Oaliphatic).

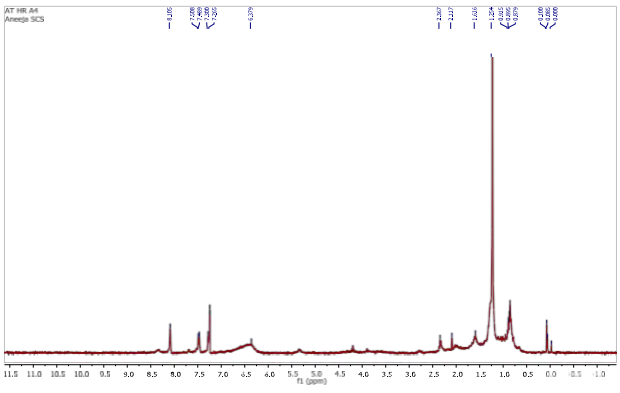


Fig 5. ^{1}H NMR Spectrum of the Compound

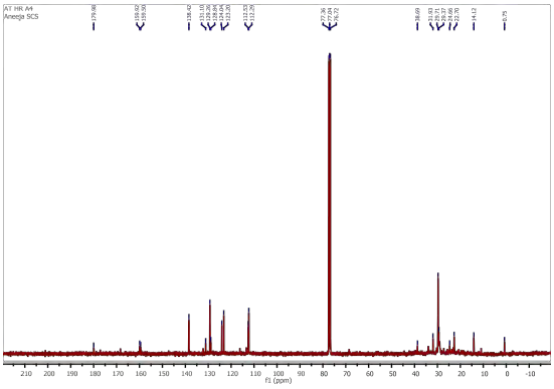


Fig 6. ^{13}C- NMR Spectrum of the Compound

Figures 5 and 6 give the NMR spectra of the compound. The ^{1}H- NMR spectrum of the compound shows the peaks (ppm) at 8.10 (s) (CHO), 7.48 (d), (ar H), 7.26 (solvent $CDCl_3$) 6.37 (s) (CH=C), 2.36 (s), 2.11 (s), 1.61 (s), 1.25 (s), 0.89 (t), 0.08 (t) (aliphatic hydrogen). From ^{13}C-NMR spectroscopy, we obtained peaks (ppm) at 170.98 (C=O), 150.5, 130.4, 128.8, 123.2 (ar C), 112.2 (C=C), 36.6, 31.9, 29.37, 24.66, 22.7, 14.5 (aliphatic C).

Figure 7 shows LC-MS spectrum of the ethanol extract of the *PZ***.** The maximum molar weight obtained is 305. The most abundant peak is observed at 185. The fragmentation pattern is given in Table 1.

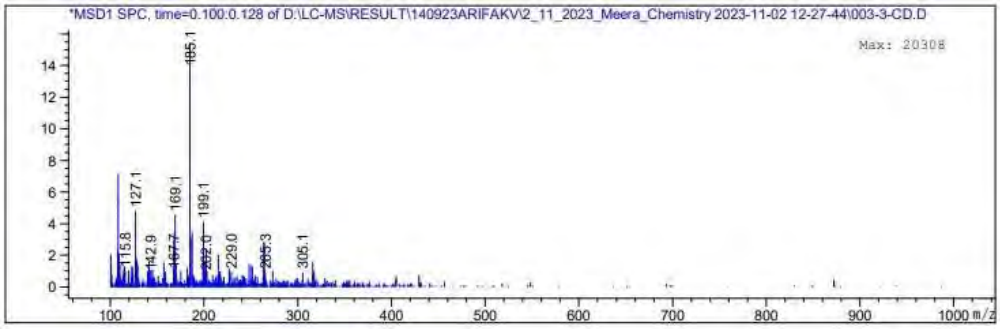
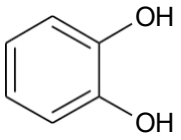
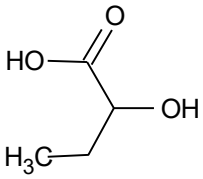
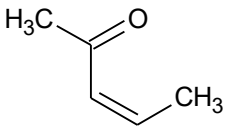


Fig 7. LC-MS Spectrum of the Ethanol Extract of the *PZ*

Table 1. The LC-MS Fragmentation Pattern of Ethanol Extract of the *PZ*

Mol. peaks	Mol. peaks Subtraction	Mass lost	Inference
414			Molecular ion peak
305	414-305	109	Loss of 
265	305-265	40	Loss of -H_2C-CH=CH-
219	305-219	86	Loss 
200	229-200	29	Loss of C_2H_5
183	265-183	17	Loss of OH
167.7	199-167.7	31	Loss of OCH_3
144	199-144	55	Loss of 
116	159-116	43	Loss of C_3H_7

As per the UV spectrum, we have a C=O and unsaturation in the molecule. From IR spectrum, we have C=C, CH_3, CH, C=O, C-N, OH and C-O groups. The mass spectrum gives the possibility of groups that are shown in Table 1.

From the literature values of various extracts of *PZ*, the following structure (Figure 8) is almost suited for our extract.

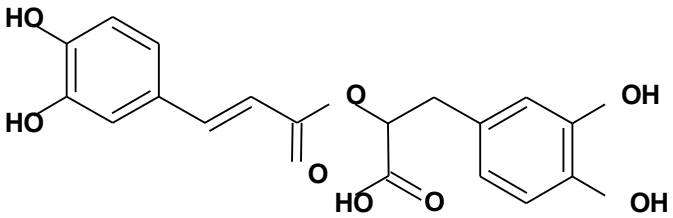


Fig 8. Structure of the Ethanol Extract of the *PZ*

The positions of the substituents are maintained at the same place. The phenolic groups may be substituted with alkyl chains. As per the mass spectrum, the molar mass is 414 (M^+ peak) and the proposed structure has the molar mass of 416.

4. Conclusion

Phytomedicines are more important in curing various chronic diseases. Plant bodies contain many chemical components in different ratios. When we analyze the therapeutic application of phytochemicals, the structural details are necessary. In this paper, the extraction of *Plectranthus zeylancius* using ethanol has been carried out. Spectroscopic techniques such as UV, IR, NMR and LCMS were used for structural elucidation. The peaks in the UV-visible spectrum show the presence of an unsaturation and carbonyl group. IR spectrum indicates the presence of C-H str of C=C, C-H str of CH_3, C-H str of CH, C=O, C=C bend, C-N, C-H bend, C-O aromatic, C-O aliphatic groups. NMR spectroscopy also suggests the existence of aliphatic and aromatic carbons and CHO group. Considering the UV, IR, NMR and LCMS spectroscopic details, the structure of the compound obtained is shown above.

5. References

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