GC-MS and HPTLC Fingerprints of Various Secondary Metabolites in the Ethanolic Extract of Coconut Shell oil

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ABSTRACT

The aim of this study was to analyze the phytochemical constituents present in the Coconut shell oil using GC-MS and HPTLC fingerprints profiles for various bioactive compounds. In GC-MS analysis the presence of many bioactive compounds were determined by different peaks with low and high molecular weight. The separation of the active constituents has been developed by HPTLC method using solvent system; Toluene:ethyl acetate: glacial acetic acid (9:1:0.2) and examined under UV 254nm, 366nm and visible light. (Vanillin- Sulphuric acid). The Coconut shell oil extract showed the presence of variety of secondary metabolites and it is expected to exhibit therapeutic properties.

Keywords: Coconut Shell Oil, GC-MS, HPTLC analysis, bioactive compounds.

INTRODUCTION

Coconut shells are widely used for enrich potting soil or covering around small plants as much in a garden setting (Prieto, 2010). The dry coconut shells contain polyphenols and organic acids. (Akhter et al., 2010) According to Zuraida et al., (2011) Coconut shell liquid smoke contain phenolic compounds such as phenols, 2-methoxy phenol (guaiacol), 3,4 - dimethoxyphenols and 2- methoxy-4- methoxyphenol. This coconut shell liquid smoke is not toxic, safe (Budijanto et al., 2008) and used in the preservation of fishes. (Jittinandana et al., 2003) Coconut shell oil has antimicrobial activity (Verma, 2012). The Coconut shell oil contains alkaloids, carbohydrates, Saponins, phenols, flavanoids, aminoacids, tannins, proteins, terpenoids, proteins, oxalate, carboxylic acid, quinines and glucosides (Dorathy, 2017). These secondary metabolites are reported to have various biological and therapeutic properties. The kernel oil in concentration of 5% to 40% inhibited bacterial activity against E.coli and Bacillus subtilis. (Oyi, 2010; Deb Mandal, 2011).

MATERIALS AND METHODS

The coconut shells were collected from the local market in Thiruvallur, Tamilnadu. The coconut shells were sundried, broken into small pieces and ground into coarse powder. Grounded coconut powder (250g) was heated in the earthen pot for a span of 3 hours giving a yield of 25 cc of oil. The oil was extracted with [1:3 v/v] ethanol for further study.

GC-MS (Gas chromatography – Mass Spectrometry) Analysis

The samples were injected into a GC-MS system consisted on a GC Clarus 500 Perkin Elmer system interfaced to a mass spectrometer and the software used is Turbomass ver 5.2 column Elite 5ms was fused with the silica capillary column (30 x 0.25 mm ID x 0.25µm thickness of film, 5%Phenyl, 95%Dimethyl Polysiloxane). Electron impact mode operated at 70 eV., Helium gas (99.999%) was used as the carrier gas at 1ml/ min of constant flow rate with injector temperature about 290°C. Electron ionization involved and the ion source temperature was 150°C. The temperature program was as follows from 50°C to 220°C with 2°C/min hold for 10min; From 220°C to 280°C with 4°C/min hold for 10 min. identification of unknown compounds were done by referring the retention times with authentic compounds and the spectral data collected from Wiley and NIST 2005 libraries.

RESULTS AND DISCUSSION

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Identification of various secondary metabolites present in the plant extracts can be done by GC-MS (Al-Haquil et al., 2015, Payum., 2016). Various phytochemicals have been identified to have a wide range of activities, which may help in protection against chronic diseases (Liu, 2003). Many bioactive compounds are present in the ethanolic extract of Coconut shell oil. The chromatogram was shown in Fig.1. The active compounds with their peak names, their molecular formula, molecular weight (mw), retention time, peak area and % of peak area are exhibited in Table-1. The presence of 20 phytoconstituents was detected in the ethanolic extract of Coconut shell oil. The HPTLC analysis were done, densitometric chromatogram, peaks and Rf values are obtained for solvent extracts after scanning at UV 254nm and 366nm solvent extracts after scanning at UV 254nm and 366nm and VS reagent. (Table -2). The HPTLC images of Coconut Shell oil shown in Fig.2 indicate that all sample constituents were clearly separated without any tailing and diffuseness. The average peak area compared with the total area and calculated for the relative percentage amount of each component. The spectrums of the separated
components were compared with the spectrum of NIST library databases. The identification of intense bands obtained from HPTLC profile showed unknown compounds. It is evident that 4 spots indicating the presence of at least 4 components in ethanol extract at 254nm and VS reagent. About 8 spots can be identified in 366nm. The HPTLC fingerprint of Coconut shell oil at 254nm shows 7 components out of which the compounds with Rf values 0.33, 0.43, 0.79, 0.94 were found to be more

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Rf Values</th>
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<tbody>
<tr>
<td>UV 254nm</td>
<td>UV 366nm</td>
</tr>
<tr>
<td>Toluene: Ethyl acetate : Glacial acetic acid (9:1:0.2)</td>
<td>0.97 Dark green</td>
</tr>
<tr>
<td>0.97 Dark green</td>
<td>0.97 Blue</td>
</tr>
<tr>
<td>0.85 Dark green</td>
<td>0.89 Violet</td>
</tr>
<tr>
<td>0.50 Dark green</td>
<td>0.75 Violet</td>
</tr>
<tr>
<td>0.39 Dark green</td>
<td>0.60 Violet</td>
</tr>
<tr>
<td>0.39 Dark green</td>
<td>0.50 Blue</td>
</tr>
<tr>
<td>0.45 Violet</td>
<td>0.41 Blue</td>
</tr>
<tr>
<td>0.39 Blue</td>
<td>0.45 Violet</td>
</tr>
<tr>
<td>0.45 Violet</td>
<td>0.39 Blue</td>
</tr>
</tbody>
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Figure 2: photo documentation under UV and VS reagent.

Figure 3: HPTLC finger print of Coconut shell oil at 254nm.
predominant as the intensity of area was 8977.3, 27183.3, 12181.1 and 4540.6 Area Unit respectively as depicted in Table 3 and the remaining components were found to be very less in quantity as the values of AU and peaks were less as shown in Fig.3. Comparatively the densitometric results of Coconut shell oil made at 366nm exhibited 4 components with Rf value ranging between 0.24 to 0.94, the intensity of area is between 724.3 to 2855.1AU (Table 4). This study revealed more number of peaks and area, when the solvent extracts were scanned at 254nm than at 366nm (Fig.4). The number of peaks and Rf values differ as per the qualitative variations of the components.

CONCLUSION
The ethanol extract of Coconut shell oil has many phytoconstituents. It represents the chemical compounds of the extract and it can be used as a source of ailments by traditional practitioners. Chemical fingerprints were obtained from the chromatographic techniques and also used as a tool for authentication and identification of plant products. The results obtained from the GC-MS and HPTLC fingerprint profiles could be useful in the authentication and quality control of the drug to ensure accuracy in therapeutics.

REFERENCES
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