DEVELOPMENT OF TLC AUTOGRAFICALLY GUIDED GC-MS PROFILE OF MAJOR PHYTOCHEMICAL CONSTITUENTS FOR COMPARATIVE ASSESSMENT IN DIFFERENT EXTRACTS OF AGERATUM CONYZOIDEZ L.

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ABSTRACT

Objective: In present study, a comparative study of TLC profile of three extracts (Dichloromethane, Methanol and n-Hexane) has been carried out for visible screening of phyto-chemical constituents. In these extracts (medium polar, polar and non polar) of A. conyzoides L( aerial part), eleven phyto-chemical constituents are assessed by GC-MS in different concentrations.

Methods: 1g of powdered A. conyzoides L. (aerial part) were soaked overnight separately in 10 ml each of DCM, methanol and n - hexane. The plant extracts were continuously stirred for 6 hrs and kept up to 18 hrs at room temperature and filtered. The solution so obtained is subjected to TLC and GC-MS study.

Results: In TLC study a number of different bands appeared at 254nm & 366nm. After derivatization, in white light there are nine bands are present.

Conclusion: The comparative study of GC-MS shown that the concentration of phyto - constituents germacrene-D, caryophylllinoxide, precocene-I, citral and trans - squalene in these three extracts are found in the order of n- hexane > DCM > methanol. It has been concluded from this study that quantitative comparative assessment of important phyto-constituents is useful for researchers to select a particular enriched extract for therapeutic efficacy.

Keywords: Ageratum conyzoides L., Phyto-chemical constituents, trans -Squalene, β-Farnesene, TLC, GC-MS.

INTRODUCTION

Agaratum conyzoides L. is most common and widely spread over the world, especially in the tropical and subtropical regions. It is mainly found in West Africa and some parts of Asia as well as South America. The medicinal plant is annual branching herb which grows up to 1 m in height.

The stems and leaves are covered with fine white hairs while the leaves are ovate and up to 7.5 cm long. The flowers are in different colour from purple to white, less than 6 mm across and arranged with terminal inflorescence. The fruits are achene and easily dispersed because of its propagation it becomes a weed and causes dispersed because of its propagation it becomes a weed and causes

The whole plant is only used for medicinal purposes and has a long history in the folk medicine of different countries. A. conyzoides, a widespread weed in Colombia and in other countries, has been used in folk medicine to cure several diseases and also as an insecticide. These products promote physiological changes in the insects that include precocious metamorphosis, sterilization, inhibition of sex pheromone production, embryo genetic damage, and interrupted circadian feeding rhythms or diapause induction [1-3]

MATERIAL AND METHODS

Collection of plant material

The aerial part of the plant A. conyzoides L. was collected around Itawa, Uttar Pradesh. The medicinal plant is identified and authenticated by Biosciences Division of Institute of Pesticide Formulation Technology (IPFT), Gurgaon, Haryana.

Sample preparation

The aerial part of the plant was dried in shade to avoid loss of essential oils. Dried plant part was powered in a mixer grinder. Powdered drug was extracted from solvents range from polar to non polar i.e. dichloromethane, methanol and n-hexane in the ratio of 1:5 (one part drug: five part solvent) by hot extraction method for six hours. The temperature of soxlet extractor is 40 oC. All crude extracts were filtered and concentrated in vacuum at 40 oC using rotatory evaporator. The concentrated extract was then dried aseptically with the help of drier.

Thin Layered Chromatography Methodology

1g of powdered A. conyzoides L. (aerial part), were soaked overnight separately in 10 ml each of DCM, methanol and n - hexane. The plant extracts were continuously stirred for 6 hrs and kept up to 18 hrs at room temperature and filtered. The solution so obtained is subjected to TLC and GC-MS study.

Gas Chromatography–Mass Spectroscopy Methodology

For GC-MS analysis of the extracts GC-MSD Shimadzu (Mass Selective Detector, GC-QP 2010 plus MSD model) equipped with DB-5MS fused silica capillary column (Agilent J & W GC column, 5% Phenylated methyl silicone, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness) was used. Analysis was carried out using oven programming of initial temperature 50 oC for 2 min followed by a ramp rate of 20 oC /min up to a temperature of 130 oC followed by 12 oC /min ramp to a temperature of 180 oC with a hold time of 10 min. The injector temperature was set at 280 oC in split less mode; solvent delay time was given as 2 min followed by a temperature of 180 oC with a hold time of 10 min. The interface and ion source temperatures were set at 280 oC and 250 oC, respectively. MS Quadruple temperature was set at 150 oC, an emission current of 300 μA, lower vacuum 3.0e + 000 Pa and upper vacuum < 1.0e - 004 Pa. The instrument was operated in Electron Impact Mode (EI) with electron energy 70 eV, carrier gas helium was used at a flow rate of 1 mL/min. For confirmation of analytes analysis was done by SIM mode. The compounds were identified by comparing the mass
spectral data with that of in-house library already stored in a compact library of chemical substances (NIST library).

RESULT AND DISCUSSION

Phyto-chemical screening

The preliminary phytochemical screening of plant aerial part in three extract was carried out as per methods and tests by Harborne J. B. [4]. The analysis showed that the presence of bioactive compounds like flavonoids, alkaloids, cumarins, essential oils, terpenes and tannins. The main chemical constituents present in aerial part is 7-dimethoxy-2,2-dimethylchromene, 6-demetoxyageratotochromene, 6-vinyl-demethoxysqualene. Out of eleven phytochemicals, five phyto-constituents are present in the plant part. In this analysis, we have detected a number of phyto-constituents.

endoborneol, endo-bornyl-acetate, ethyl-eugenol, ethyl-vanillin, farnesol, friedelin, hydrogen-cyanide, hexadecanoic acid, Kaempferol, Kaempferol-3,7-diglucoside, Kaempferol-3-O- rhamnosylglucoside, linoleic acid, quercetin, quercetin-3,7-diglucoside and quercetin-3-O-rhamnosylglucoside [5, 6, 7-10].

Comparative study of TLC chromatogram

On comparative study of TLC chromatogram, it has been observed that a number of different bands appeared at 254nm & 366nm. After derivatization, in white light there are nine bands are present in these three extracts, shown in Figure 1. There are some bands which have similar Rf values in these three extracts as shown in Table 1.

This may lead to identify and estimate these different chemical constituents by GC-MS.

Table 1: Rf Values of three different extracts of aerial part.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Visualization / Detection (Rf Values)</th>
<th>UV 254 nm</th>
<th>UV 366 nm</th>
<th>White Light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Track 1</td>
<td>0.52, 0.45, 0.54, 0.60</td>
<td>0.07, 0.12, 0.29, 0.33, 0.48, 0.57</td>
<td>0.25, 0.47, 0.56, 0.61</td>
<td>0.20, 0.65</td>
</tr>
<tr>
<td>Track 2</td>
<td>0.34, 0.62</td>
<td>0.07, 0.29, 0.33, 0.48, 0.57</td>
<td>0.20, 0.65</td>
<td></td>
</tr>
<tr>
<td>Track 3</td>
<td>0.22, 0.34, 0.47, 0.54, 0.60</td>
<td>0.07, 0.12, 0.22, 0.33, 0.48, 0.57, 0.71</td>
<td>0.28, 0.35, 0.63</td>
<td></td>
</tr>
</tbody>
</table>

GC-MS analysis

GC – MS analysis was carried out on three extract (DCM, methanol and n-Hexane) of A. conizoides L. After analysis, it is found that eleven major phyto-constituents present in the aerial part of medicinal plant. In this analysis, we have detected a number of phyto-constituents present in the plant part i.e., (+) β - fenebrene, germacrone - D, trans - (β) caryophyllene, β - farnesene, caryophylloxide, precocene-I, precocene-II, hexadecanoic acid methyl ester, α - linolenic acid, citroinol and trans- squalene. Out of eleven phyto-constituents, five phyto-constituents namely germacrone - D, caryophylloxide, precocene-I, citroinol and trans- squalene are found having highest concentration in n - hexane extract as compared to DCM and methanol extract. The compounds (+) β - fenebrene, hexadecanoic acid methyl ester and α - linolenic acid are in higher concentration present in alkoholic extract while trans- (β) caryophyllene, β - farnesene and precocene-II are present in more concentration in DCM than the methanolic and n - hexane extract.

In dichloromethane extract, the highest peak area (63.05 %) is present in precocene-II (RT: 12.882) while the lowest peak area (0.38 %) present in hexadecanoic acid (RT: 17.127). In alcoholic extract, the highest peak area (57.08 %) are present in precocene-II (RT: 12.883), however, the lowest peak area (0.49 %) present in citroinol (RT: 21.127). In n-hexane extract the highest peak area (51.70 %) are present in precocene-II (RT: 12.886) and the lowest peak area (0.51 %) present in (+) β - fenebrene (RT: 9.470).

The overall highest peak area present in these three extracts (63.05 %) is precocene-II (RT:12.882) and the lowest peak area (0.38 %) present in hexadecanoic acid (RT:17.127) in DCM extract. The detail results of GC-MS analysis has been given in Table 2 and the total ion chromatography (TIC) showing the peak identity of the compounds identified have been shown in Figure 2, 3 and 4.

Table 2: Details of Phyto-constituents identified from three extracts of aerial part of A. conizoides L by GC-MS

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phyto-constituents</th>
<th>DCM extract</th>
<th>Methanol Extract</th>
<th>Hexane extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Retention time (%)</td>
<td>Peak area (RT)</td>
<td>Retention time (%)</td>
</tr>
<tr>
<td>1.</td>
<td>(+) – (β) - Fenebrene</td>
<td>9.483</td>
<td>0.59</td>
<td>9.480</td>
</tr>
<tr>
<td>2.</td>
<td>Germacrone-D</td>
<td>9.884</td>
<td>0.67</td>
<td>9.880</td>
</tr>
<tr>
<td>3.</td>
<td>trans – (β) - Caryophyllene</td>
<td>10.242</td>
<td>3.98</td>
<td>10.240</td>
</tr>
<tr>
<td>4.</td>
<td>β - Farnesene</td>
<td>10.085</td>
<td>1.07</td>
<td>10.376</td>
</tr>
<tr>
<td>5.</td>
<td>Caryophylloxide</td>
<td>12.086</td>
<td>1.60</td>
<td>12.082</td>
</tr>
<tr>
<td>6.</td>
<td>Precocene-II</td>
<td>12.882</td>
<td>63.05</td>
<td>12.853</td>
</tr>
<tr>
<td>8.</td>
<td>Hexadecanoic acid methyl ester</td>
<td>17.127</td>
<td>0.38</td>
<td>17.110</td>
</tr>
<tr>
<td>9.</td>
<td>α - Linolenic acid</td>
<td>20.924</td>
<td>0.24</td>
<td>20.909</td>
</tr>
<tr>
<td>10.</td>
<td>Citroinol</td>
<td>21.228</td>
<td>0.97</td>
<td>21.127</td>
</tr>
<tr>
<td>11.</td>
<td>trans - Squalene</td>
<td>38.104</td>
<td>13.18</td>
<td>38.089</td>
</tr>
</tbody>
</table>
CONCLUSION

The comparative study of GC-MS shown that the concentration of phyto-constituents germacrene-D, caryophyllloxide, precocene-I, citrinol, and trans- squalene in these three extracts are found in the order of n-hexane > DCM > methanol. The concentration of trans – (β) caryophyllene and β- farnecene are in order of DCM > Hexane > Methanol while the hexadecanoic acid methyl ester and ε-linolic acid is having more concentration in methanol as compared to n-hexane and DCM. It has been also analyzed that the concentration of (+) - (β)-farnesene and precocene-II are found in the order of Methanol > DCM > Hexane and DCM > Methanol > Hexane, respectively.

It has been concluded from this study that quantitative comparative assessment of important phyto-constituents is useful for researchers to select a particular enriched extract for therapeutic efficacy.

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REFERENCES