CHAPTER 2

PHYTOCHEMICAL STUDIES ON THE ROOT EXTRACT OF SYNEDRELLA NODIFLORA

2.1 Introduction:

*Synedrella* is a genus of flowering plants in the daisy family and it has three species. *Synedrella* weed is usually found in flower beds, along road sides and in crops and plantations. It grows best where the soil is moist and fertile, and where there is plenty of light. It is adapted to many environments. It is particularly well adapted to the partial shade found under jute and plantation crops tea, coffee, banana, cocoa and rubber. It is quite palatable to livestock, and so is not a problem in pasture. In some countries it is fed to pigs.

*Synedrella nodiflora* (L.) Gaertn (*Asteraceae*) – node weed is an annual herb which grows to about 2m high. It is a native tropical American weed but now dispersed pan-tropically and occurring throughout the West African sub region and found in the plains of India, Andaman, Nigeria, and can also be found over a wide range of locations worldwide. There have been reports that this plant has medicinal value [1], potent anti-inflammatory effect, effective analgesic and antipyretic activities in rodents [2]. There are also reports that it has insecticidal activity and that is now been traditionally used in Ghana to control insect pests of stored grains and legumes. This is confirmed by the study [3] that observed the antifeedant activity of *S. nodiflora* against *Pieris raphae* (an insect). The leaves are used as poultice for sore rheumatism and juice of the leaves is used for earache [4]. In Ghana infusion of leaves is taken as laxative; leaf sap is used in Cango for mouth
affections and is rubbed on gums for tightening. In Malaya it is used for poulticing sore legs and for headache after confinement and the sap is put in to ear for earache in Indonesia, leaf sap is used for stomachache and rheumatism, roots are pounded and cooked and decoction is given as cough mixture in Tganyika [5]. Insecticidal activity of aerial parts of *S. nodiflora* on *Sapodeptera latura* was reported [4]. In Ghanian traditional medicine, the aqueous extract of the whole plant is drunk for the treatment of epilepsy, whilst the leaves are used for the treatment of hiccup and threatened abortion [6]. The plant is used extensively in Nigeria for cardiac troubles, wounds and for stopping bleeding [7]. In agreement with its traditional uses, [1] showed potent anti-inflammatory effects of ethanolic extract of the aerial parts. Analgesic properties of the whole plant have also been demonstrated in the hot plate and acetic acid inducing writhing models of pain [2]. The toxicological effect of *S. nodiflora* was evaluated in albino rats using aqueous crude extract of the plant. The herb was tested for its antimicrobial potential as *Asteraceae* are a successful family with several defense chemicals [8].

Ghayal and coworkers [9,10] detected acid, esters and alcohols by GC-MS of *S. nodiflora* and a study on the impact of its leaf leachates on germination and seedling growth of radish and mustard. Prekay and coworkers [11] have evaluated the effects of dietary supplementation of node weed *S. nodiflora* on toxicity of copper and lead in guinea pigs. A new triterpenoid Saponin, Nodifloside A was isolated from the 85% ethanol extract of the whole plant of *S. nodiflora* by Yang and coworkers [12]. Essential oils from leaves of *S. nodiflora* were analyzed by Aalbersberg and coworkers [13]. Antidiarrhoeal and hypoglycemic potential of the methanolic extract of *S. nodiflora* leaves were investigated by Ronok and coworkers [14]. Patrik and coworkers identified the anticonvulsant and related
neuropharmacological effects of the whole plant extract of *S.nodiflora* [15]. Antinociceptive, antimicrobial, antioxidant, ethopharmacological analysis, analgesic effects also have been studied [16-20]. Structures of compounds obtained from *S.nodiflora* are given in the Figure 2.1.

*Figure 2.1. Structures of compounds from *S.nodiflora***

<table>
<thead>
<tr>
<th>Stigmasterol</th>
<th>δ-Cadinene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydrostigmasterol</td>
<td>α-Cadinol</td>
</tr>
</tbody>
</table>

2.2 Present Work:

The study presented in this section comprise of the phytochemical investigation of roots of *S.nodiflora*. This includes the extraction of the dried plant material using different solvent systems, the fractionation and isolation of the chemical constituents, their purification and characterization of the individual compounds using various spectral techniques.

2.3 Scope of the work:

From the literatures on the genus *Synedrella* it is very much evident that all the species belonging to this genus are rich sources of a variety of chemical
compounds with exceptional attributes. Not many are thoroughly studied and there is much more to be explored regarding their chemistry and biological activity. This makes the plant more interesting and this study reasonable. The already reported compounds from *S. nodiflora* mainly belong to sterols, triterpenes, flavanoids and phenolics with interesting structural backbone and are found to possess remarkable properties. The plant shows marked regional variations with respect to the compounds present in them, a novelty in the chemical profile or a change in the amount of useful molecules can always be expected.

2.4 **Plant Material:**

The roots of *S. nodiflora* were collected from Malappuram district, Kerala. It was identified by Dr. A.K Pradeep, Department of Botany, University of Calicut. A voucher specimen was deposited in the Herbarium, Department of Botany, University of Calicut.

2.5 **Materials and Methods:**

All melting points were determined on Toshniwal melting point apparatus and are uncorrected. UV spectra were obtained in a JASCO UV spectrometer. IR spectra (KBr) were taken on a JASCO FT-IR spectrometer. GC/MS analysis was done with Varian 4000 GC-MS. Column chromatography were carried out using Silica gel (100-200, mesh size, MERCK). The columns were prepared as slurry with suitable solvents and a gradient elution was carried out by mixing of solvents with different polarity. Silica gel G Merck for preparative thin layer chromatography using Stahl apparatus.

**Spray reagents in TLC:**
1. **20% aqueous sulphuric acid (20% H$_2$SO$_4$)**

20% aqueous sulphuric acid was prepared, the sprayed plate was heated to 110°C until spots are visible. With this reagent, the terpenoids developed brown, pink, purple or yellow colour.

2. **Liebermann-Burchard Test**

Dissolve one or two crystals of solid in dry CHCl$_3$ in a dry test tube. Add several drops of acetic anhydride and then 2 drops of concentrated H$_2$SO$_4$ and mix carefully. Development of pink colour indicates the presence of triterpenoids and sterols.

2.6 **Experimental:**

**Extraction:**

The roots of *S.nodiflora* were shade dried. Coarsely powdered 5kg of the plant material and extracted successively with 3X7L of light petroleum ether and acetone.

The extraction was carried out in a round bottom flask by boiling the material in the respective solvents with a water condenser, over a water bath. Refluxed the material until the solvent started to boil and the hot content was left standing overnight. Then filtered and collected the extract and added fresh solvent to residue. The process is repeated four times to complete the extraction. The combined extract was collected and reduced to 200 ml under vacuum.

**Fractionation:**
The extract was adsorbed on 250 gm silica gel. This was then loaded on a preparative column. (Dimension usually being 7cmX100cm; dX1) The column was packed with silica gel as the stationary phase which was wetted using petroleum ether to achieve least polarity to the mobile phase during the beginning of elution. The mobile phase for elution was fixed based on the TLC analysis. The elution was carried out by gradient elution technique; the gradation of the mobile phase polarity was achieved by homogenous mixing the petroleum ether and ethylacetate of different polarity. The different fractions collected were again analysed using TLC and the similar fractions were combined and concentrated to 50 ml. These fractions were stored under refrigeration for further analysis.

**Petroleum ether extract**

From the petroleum ether extract a solid crystalline substance (SB) separated out. It was filtered, washed with petroleum ether several times. It melted at 290°C. TLC showed a pink colour on spraying 20% H₂SO₄ and heating to 100°C. This compound is identified by different spectroscopic techniques.

**Acetone extract**

The concentrated acetone extract after removal of solvent, yielded 60g of dark residue. This was then dissolved in 120 ml hot acetone and adsorbed in 200 g silica gel. It was then loaded on a preparative column using silica gel wetted with petroleum ether as the stationary phase. The fraction on elution with petroleum ether and ethyl acetate in the ratio 8:1 on concentration yielded a yellow syrupy liquid (SO). It showed large number of spots on TLC examination. It was analyzed by GC-MS.
On eluting the column with petroleum ether ethyl acetate in 5:1 ratio a white solid was obtained (SW) which melted at 144°C. TLC showed a pink spot on spraying 20% H_2SO_4 and heating to 100°C.

2.7 Results and Discussion:

Isolation of compounds

Characterization of SB

The solid obtained from the petroleum ether extract has a melting point of 290°C. TLC showed a pink spot on spraying with 20% H_2SO_4 when heated to 100°C. IR spectrum has characteristic peaks at 3408.57 cm\(^{-1}\), 2956.34 cm\(^{-1}\), 2917.77 cm\(^{-1}\), 2849.31 cm\(^{-1}\), 1736.58 cm\(^{-1}\), 1633.41 cm\(^{-1}\), 1514.81 cm\(^{-1}\), 1463.7 cm\(^{-1}\), 1166.72 cm\(^{-1}\), 1024.02 cm\(^{-1}\), 728.86 cm\(^{-1}\). Mass spectrum (Figure 2.2.) showed the molecular ion peak at 468 and other fragmented peaks at 453, 393, 209, 229, and 205. The proton NMR (Figure 2.3.) of this compound has a one proton signal at δ 4.5 corresponding to an olefinic proton and another one at δ 5.44 corresponding to proton at C-3 which has α-orientation. The \(^{13}\)C NMR (Figure 2.4.) corresponds to that reported for bauerenyl acetate. [21]. This structure is supported by its mass spectrum which has M+ peak at m/z = 468 and base peak at m/z = 289.
Characterisation of SO

The compound SO showed different coloured spots on TLC proving it to be a mixture. UV in methanol shows characteristic peaks at 226, 278 and at 318 nm. IR shows characteristic peaks at 3415 cm$^{-1}$, 2918.73 cm$^{-1}$, 2847 cm$^{-1}$, 1712.48 cm$^{-1}$, 1456.96 cm$^{-1}$, 1244 cm$^{-1}$, 723 cm$^{-1}$. GC-MS shows the presence of the compounds given in the Table 2.1.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>%</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.34</td>
<td>1-Hexadecanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>2</td>
<td>28.055</td>
<td>Tritetracontane</td>
</tr>
<tr>
<td>3</td>
<td>1.363</td>
<td>2-Hexyldecanol</td>
</tr>
<tr>
<td>4</td>
<td>1.136</td>
<td>Z,E-2,13 Octadecadien-1-ol</td>
</tr>
<tr>
<td>5</td>
<td>5.76</td>
<td>Hexatriacontane</td>
</tr>
<tr>
<td>6</td>
<td>1.949</td>
<td>3-Ethyl-3-hydroxyandrostan-17-one</td>
</tr>
<tr>
<td>7</td>
<td>4.188</td>
<td>1-Heptadecanol</td>
</tr>
<tr>
<td>8</td>
<td>3.577</td>
<td>3,5,24-Trimethyltetracontane</td>
</tr>
<tr>
<td>9</td>
<td>3.758</td>
<td>2-Hexyl-1-octanol</td>
</tr>
<tr>
<td>10</td>
<td>1.744</td>
<td>Tetratetracontane</td>
</tr>
<tr>
<td>11</td>
<td>1.281</td>
<td>Heptacosane</td>
</tr>
<tr>
<td>12</td>
<td>3.843</td>
<td>Tetrapentacontane</td>
</tr>
</tbody>
</table>

**Characterisation of SW (β-Sitosterol)**

SW was isolated from the acetone extract on elution with a 5:1 mixture of petroleum ether and ethyl acetate as a white powdery substance. On recrystallization from ethyl acetate it gave colorless needles with a melting point 144°C. Leibermann Burchard reagent gave a play of colors indicating that it was a sterol. EI-MS of this compound showed M+ at m/z 414 and base peak at m/z 55. IR showed the presence of hydroxyl group (broad absorption band at 3441.4 cm⁻¹), bands due to gem dimethyl group (doublet at 1383.1 & 1385.1 cm⁻¹), -CH stretching and bending bands at (2981, 2816, 1464, 1470 cm⁻¹) were also observed in the spectrum. ¹H-NMR was quite comparable with that of β-Sitosterol. A direct comparison of melting point with authentic sample established its identity. The derivatives, acetate (m.p 125°C) and benzoate (m.p 145°C) of the compound prepared were found to be identical with β-Sitosterol acetate (m.p 127°C) and β-Sitosterol benzoate (m.p 145°C) [22]. Mixed melting point with an authentic sample was undepressed.

1) Acetylation of SW
100mg of SW was refluxed with 3 ml pyridine and 1 ml acetic anhydride for 2 hours. Poured the reaction mixture into cold water, filtered washed with water and then recrystallized from petroleum spirit. Melting point of compound 133°C.

2) Benzoylation of SW

100 mg of SW was refluxed with 2 ml freshly distilled benzoyl chloride and 5 ml pyridine on a sand bath for 2 hours. The reaction mixture was then poured into cold water, filtered, washed with water and recrystallized from benzene. The compound melted at 144°C.
2.8 References:


