Research Article

FREE RADICAL SCAVENGING, GC-MS AND FT-IR ANALYSES OF DIFFERENT SOLVENT EXTRACTS OF LEAF, STEM AND ROOT OF Oxystelma esculentum R.Br.

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Abstract

Oxystelma esculentum distributed in the southern part of India particularly in Tamil Nadu has potential medicinal properties and it is used as diuretic, anthelmintic, oxytocic and laxative. Total phenol content and Total flavonoids content. The free radical scavenging activities of IC$_{50}$ values of Total antioxidant and 2,2-Diphenyl-1-picrylhydrazyl DPPH assay. The leaves were sequentially extracted based on the polarity viz., petroleum ether, chloroform, ethyl acetate, acetone and methanol. The methanol extract showed the presence of all phytoconstituents studied. The GC-MS analysis of the methanol extract revealed the presence of five compounds and FT-IR analysis. 5-Allylsulfanyl-1-(4-methoxy-phenyl)-1H-tetrazole is an major compounds and 1-(2-Ethyl-1,3)dithian-2-yl)-3-methyl-butanol-1-ol may be responsible for free radical scavenging activities.

Key words: Oxystelma esculentum, Free radical scavenging, GC-MS and FT-IR analysis of leaf, Stem and root extract.

1. Introduction

Medicinal plants are represent a rich source of antimicrobial and antioxidant agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et al., 1996). A wide range of medicinal plant parts are used for extract as raw drugs and they possess varied medicinal properties. The different parts used are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in quantities and traded in the market as the raw material for many herbal industries (Uniyal et al., 2006). Bioactive compounds from plants possess various applications in food, nutraceutical and pharmaceutical industries (Schwarz et al., 2001), due to their antimicrobial, antioxidant and cytotoxic properties. Various phytoconstituents and their potential industrial applications have been reported previously (Denny and Buttriss, 2007).

Natural products represent a major source of chemical diversity that includes potentially innovative therapeutic agents for treating various conditions, including infectious diseases (Mohamed et al., 2014). It was estimated that two-thirds of the world’s population continues to rely mainly on traditional plant based medical remedies due to the limited availability and affordability of pharmaceutical medicines (Puri et al., 2012). In developing countries, communities rely heavily on traditional herbal medicines in order to meet their primary health care needs. In many industrialized countries herbal medicines are

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gaining alternative and complimentary therapies. Some of the plants are used as food or medicine. These plants are exhibit a wide range of biological and pharmacological activities such as anti-inflammatory, diuretic, oxytocic, laxative, antihypertensive, anti-diabetic and anti-microbial functions. The secondary metabolites of plants are provides humans with numerous biological active products which has been used extensively as drugs, foods, additives, flavors, insecticides, colorants, fragrances and chemicals (El Abdouni Khiyari et al., 2014).

Antioxidant capacity must be evaluated by a number of methods to take into account the different modes of action of a particular antioxidant (Clardy and Walsh, 2004). GC-MS and FTIR has played an important role in pharmaceutical analysis in recent years (Tagboto and Townson, 2001; Koduru et al., 2006; Chen et al., 2001; Movasaghi et al., 2008), recently, spectroscopy has emerged as one of the major tools for biomedical applications and has made significant progress in the field of clinical evaluation. Research has been carried out on a number of natural tissues using spectroscopic techniques, including FT-IR spectroscopy.

2. Materials and Methods

Collection of plant material

Oxystelma esculentum (leaves, stem and roots) were collected from Annamalai University campus, Annamalainagar, chidambaram (latitude 11°23’17 n; longitude 79°42’57 E) Cuddalore district, Tamilnadu, India during month of June, 2013. Herbarium was deposited in department of botany, Annamalai University, (voucher specimen No. AUBOT#256). The collected specimens were washed with tap water, then surface sterilized with 10 per cent sodium hypochlorite solution, rinsed with sterile distilled water and allowed to shade dried under room temperature. The samples were ground into fine powder using an electric blender.

Determination of total phenolic content

Determination of total phenolic content was carried out following the Folin - Ciocalteu method by Rao (1963). One mL solution containing (1 mg/mL) was added volumetric flask. One mL of Folin - Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min; 7.5 % of 0.75 mL of sodium bicarbonate solution was added and mixed thoroughly. The samples were measured spectrophotometrically at 765 nm using spectrometer after 90 min at 22 °C. The amount of total phenolic were determined as Gallic acid and equivalent and expressed as mg GAE/G dry weight.

Determination of total flavonoid content

The flavonoids content was determined by Aluminum dichloride method using Rutin as a reference compound (Deepa et al., 2013). This method based on the formation of a complex flavonoids - aluminum having the absorptivity maximum at 415 nm, after remained react at room temperature for 30 min. Briefly, 0.5 mL of each extracts (1:10 g/mL) in methanol was separately mixed with 1.5 mL of methanol, 0.1 mL of 10 % aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The amount of total flavonoids was determined as mg Rutin/g.

Determination of Total antioxidant activity

Total antioxidant activity of different crude extracts of Oxystelma esculentum was determined according to the method of (Moussa et al., 2011). Briefly, 0.3 mL of samples was mixed with 3.0 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molydate). Reaction mixture was incubated at 95 °C for 90 min under water bath. Absorbance of all the sample mixture was measured at 695 nm. Total antioxidant activity was expressed as the number of equivalents of ascorbic acid.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The DPPH scavenging activity of different extracts of Oxystelma esculentum was measured according to the procedure described by Wan et al. (2011). Radical scavenging activity of plant extracts against stable DPPH radical was determined spectrophotometrically. The colorimetric change (from deep - violet to light yellow), 1 mL of each solution added to 3 mL of 0.004 % methanolic DPPH free radical solution.
After 30 mins, the absorbance of the preparations were taken at 517 nm by a UV spectrophotometer (Hitachi-U-20) which was compared with the corresponding absorbance of standard ascorbic acid concentrations (100, 150, 200, 250 and 300 µg/mL). Methanol was used to zero the spectrophotometer absorbance of the DPPH radical without antioxidants. The control, was measured special care was taken to minimize the loss of free radical activities of DPPH radical stock solution was calculated by the following equation.

\[
\text{Radical scavenging activity (\%)} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100
\]

Extracts concentration providing 50% inhibition IC\text{50} calculation.

**GC–MS Spectrometry**

Gas chromatography (GC) analysis was carried out using Varian 3800 gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatograph was fitted with DB 5 MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µM). The injector temperature was set at 280 °C, and the oven temperature was initially at 45 °C then programmed to 300 °C at the rate of 10 °C/min and finally held at 200 °C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 mL/min. One microlitre of the sample (diluted with acetone 1:10) was injected in the split mode in the ratio of 1:100. The percentage of composition of the compound was calculated by the GC peak areas. GC–MS analysis of compound was performed using Varian 3800 gas chromatography equipped with Varian 1200 L single quadrupole Mass Spectrometer. GC conditions were the same as reported for GC analysis and the same column was used. The mass spectrometer was operated in the electron impact mode at 70 eV. Ion source and transfer line temperature was kept at 250 °C. The mass spectra were obtained by centroid scan of the mass range from 40 to 100 amu. The compounds were identified based on the comparison of their retention time (RT), mass spectra of WILEY, NIST library data of the GC-MS system.

### 3. Result and Discussion

The results of total phenolic and flavonoids content of leaf stem and root extracts of *Oxystelma esculentum* are presented in Fig – 1 and 2. The total phenols (1.67 ± 0.22) mg gallic acid equivalent (GAE/g) and flavonoids (6.38 ± 0.50) mg rutin equivalent (CE/g) were found to be higher in methanol extract of leaves of *Oxystelma esculentum*. However, the lowest content of phenols (0.02 mg/g extract) and flavonoids (0.41 mg/g extract) were observed in chloroform extracts of root.

**Total antioxidant activity**

The total antioxidant activity of petroleum ether, chloroform, ethyl acetate, acetone and methanol of *Oxystelma esculentum* leaves, stem and root was carried out and the results are shown in Fig - 3. The positive standard, Ascorbic acid was used. The highest total antioxidant activity was recorded with methanol extracts of *O. esculentum* followed by ethyl acetate, acetone, chloroform and petroleum ether. The IC\text{50} values of acetone extracts of this plant leaf, stem, root and L-ascorbic acid values were found to be 153, 300, 353 and 186 µg/ml.

**DPPH free radical scavenging assay**

The results of DPPH•(2,2-diphenyl-1-picyrylhydrazyl) radical scavenging activity of various extracts of leaf, stem and root of *Oxystelma* and the radical scavenging activity of the positive standard (Ascorbic acid) are presented Fig - 4. Methanol extracts of leaves, stem and root of *O. esculentum* exhibited the highest DPPH activity followed by ethyl acetate, acetone, petroleum ether and chloroform. The IC\text{50} values of methanol extracts of leaf, stem, root and L-ascorbic acid were found to be 121, 232, 255 and 170 µg/ml respectively.
Fig - 1: Total phenol content

Fig - 2: Total flavonoids content

Fig – 3: Total antioxidant activity of *O. esculentum* Leaf extracts

Fig – 4: Total antioxidant activity of *O. esculentum* stem extracts

Fig – 5: Total antioxidant activity of *O. esculentum* root extracts

Fig – 6: Scavenging effect on DPPH of leaf extract of *O. esculentum*
Table – 1: Compounds identified in methanol leaf extract of *Oxystelma esculentum*

<table>
<thead>
<tr>
<th>S.NO</th>
<th>RT</th>
<th>Chemical of compounds</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>% peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.93</td>
<td>5b- Cholestanol</td>
<td>C_{27}H_{48}O_{1}</td>
<td>388.370</td>
<td>3%</td>
</tr>
<tr>
<td>2</td>
<td>10.2</td>
<td>Dasycarpidan-1-methanol, acetate (ester)</td>
<td>C_{20}H_{26}N_{2}O_{2}</td>
<td>326.432</td>
<td>14%</td>
</tr>
<tr>
<td>3</td>
<td>12.27</td>
<td>Carda-4,20(22)-diesoline, 3-[(6-deoxy-3-0-methyl-a’-D-allopyranosyl)oxy]-1,14-dihydroxy-, (1’a,3’a)</td>
<td>C_{30}H_{44}O_{9}</td>
<td>532.665</td>
<td>18%</td>
</tr>
<tr>
<td>4</td>
<td>14.02</td>
<td>1-(2-Ethyl-(1,3)dithian-2-yl)-3-methyl-butan-1-ol</td>
<td>C_{11}H_{22}O_{52}</td>
<td>234.422</td>
<td>25%</td>
</tr>
<tr>
<td>5</td>
<td>15.45</td>
<td>5-Allylsulfanyl-1-(4-methoxy-phenyl)-1H-tetrazole</td>
<td>C_{11}H_{12}N_{4}O_{5}</td>
<td>248.304</td>
<td>40%</td>
</tr>
</tbody>
</table>

The compounds present in the methanol leaf extract of *Oxystelma esculentum* were identified by GC-MS analysis are presented in Table – 1 and Fig - 9. The active principle, Molecular Weight (MW), Concentration (%), Molecular Formula (MF), and Retention Time (RT) was presented in More than five compounds were identified in the extract being 5-Allylsulfanyl – 1 - (4 – methoxy - phenyl) -1H-tetrazole (40 %), 1- (2-Ethyl- (1,3) dithian -2-yl)-3-methyl-butan-1-ol (25%), Carda-4,20(22)-diesoline,3-[6-deoxy-3-0-methyl-a’-D-allopyranosyl]oxy]-1,14-dihydroxy-, 1’a,3’a) (18%), Dasycarpidan-1-methanol, acetate (ester) (14%), 5b- Cholestanol (3%), respectively along with other minor constituents. The identified compounds in the leaf of methanol extract of *Oxystelma esculentum*. ©2015 Published by JPS Scientific Publications Ltd. All rights reserved
The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The outcome of FT-IR functional groups were represented in FT-IR spectrum of Oxystelma esculentum plant leaves extract were observed different functional groups peaks with different intensity (Fig – 10). The broad peak observed 3365 cm\(^{-1}\) assigned to OH Stretching of Alcohols groups. The medium peak observed at 2441 cm\(^{-1}\) O-H stretching of carboxylic acid groups. 2176 cm\(^{-1}\) C = C stretching of Alkynes, 1468 cm\(^{-1}\) C-H bending of Alkanes and Alklys, 1421 C-H bending of alkenes, 1277 cm\(^{-1}\) C = O-O-C Stretching of aliphatic acetate, 1256 C-O-C sym stretching of ethers, 1130 C-O-C stretching of ethers, 1004 c-F stretching of AKYL Halides. The small peaks observed at 826, 863, 707, 673 and 617 cm\(^{-1}\). Were C-cl stretching of Alkyl halides, C-H bending of Alkenes and C-H bending of Alkynes. The strong peak observed at 946 C-H bending of Alkenes groups. 5-allyl sulfanyl – 1 - (4 – methoxy - phenyl) - 1H - tetrazole is an major compounds and 1- (2- Ethyl- (1,3) dithian-2-yl)-3-methyl-butanol may be responsible for free radical scavenging activities.
4. Conclusion
Finally, the present investigation it can be concluded that the highest extraction of phytochemicals was observed in methanol extract then petroleum ether, chloroform, ethyl acetate acetone and methanol. Moreover, GC-MS analyses showed the five compounds with variable chemical suggest the contribution of these compounds in pharmacological activities.

5. Reference


