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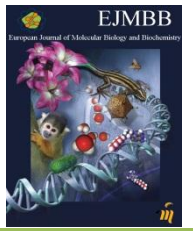

PHYTOCHEMICAL ANALYSIS OF METHANOL EXTRACT OF *OXYSTELMA ESCULENTUM* R.BR.

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ABSTRACT

The objective of the present study was to screen the phytochemicals present in the methanol extract of *Oxystelma esculentum* R.Br. The phytochemical screening of methanol extract was screened using the standard procedure for UV-Vis spectroscopic, HPLC and FTIR. The UV-Visible spectrum showed the compounds separated at the nm of 250, 300, 350, 400, 450, 502, 534, 606, 664 and 700 with the absorption 1.699, 3.568, 3.505, 3.374, 0.867, 0.509, 0.423, 0.423, 1.001 and 0.018 respectively. The qualitative HPLC fingerprint profile displayed seven compounds at different retention times. The profile displayed four compounds at different retention times of 1.867min, 2.063min, 2.797min and 3.000min. The profile displayed three prominent peaks at the retention times of 1.687min, 2.063min and 2.797min, followed by only one moderate peak at the retention time of 3.000min. The result of FTIR analysis was found the presence of functional groups such as sulphonic acid, sulphates, cycloalkanes, phenyl, primary amines, methane, alkanes, aromatic, nitrate esters, 2-halo, alkanes and lactans.

INTRODUCTION

A number of phytochemical are known, some of which include alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids and terpenoids. They do not only protect the plants but have enormous physiological activities in humans and animals. These include cancer prevention, antibacterial, antifungal, antioxidative, hormonal action, enzyme stimulation and many more. Phytochemicals are responsible for medicinal activity of plants and they have protected human from various diseases [1]. Phytochemicals are basically divided into two groups that are primary and secondary metabolites based on the function in plant metabolism. The major constituents of phytochemical are consist of carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and among others [2].

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The phytochemical constituents are playing a significant role in the identification of crude drugs also. There is widespread interest in evaluating drugs derived from plant sources. This interest mainly arises from the belief that green medicine is safe and dependable, compared to costly synthetic drugs which are invariably associated with adverse effects [3]. Natural antimicrobials have been often derived from plants, animal tissues or microorganisms. The adverse effects of the drugs available today, necessitate the discovery of new harmless pharmacotherapeutic agents from medicinal plants [4-5]. The present study looks into the fundamental scientific bases for the characterization of phytochemicals of *Oxystelma esculentum* R.Br. using UV-Visible spectroscopy, High Performance Liquid Chromatography (HPLC) and Fourier Transmission Infra Red (FTIR) spectroscopy.

MATERIALS AND METHODS

Collection of plant materials

The plant material selected for the present study is

Oxystelma esculentum R.Br. (Figure 1) belonging to the family Asclepiadaceae which was collected near new bus stand, Tirunelveli, Tamil Nadu, India, during the month of December, 2015 and identified and confirmed by the flora of the Presidency of Madras (Gamble, 1919). The collected materials were washed thoroughly with tap water to remove the sediment particles. Then the samples were brought in polythene bag to the laboratory, followed by washed using distilled water. They were stored in refrigerator for further use.

Preparation of extracts

For the preparation of different extracts, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered samples were packed in Soxhlet apparatus and extracted with methanol, chloroform, benzene and hexane for 8h separately [6].

UV-Vis spectral analysis

The methanol crude extract containing the bioactive compound was analyzed UV-Vis spectroscopically for further confirmation. The methanol crude extract of *Oxystelma esculentum* R.Br. was scanned in a wavelength ranging from 310-900nm using a Shimazdu spectrophotometer and characteristic peaks were detected [7].

HPLC Analysis

The HPLC method was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, a Rheodyne injector fitted with a 20µl loop and an auto injector SIL-10AT. A Hypersil® BDS C-18 column (4.6 × 250mm, 5µm size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1ml/min at ambient temperature (25-28°C). The mobile phase consisted of 0.1% v/v methanol (solvent A) and water (solvent B). The mobile phase was prepared daily, filtered through a 0.45µm and sonicated before use. Total running time was 15min. The sample injection volume was 20µl while the wavelength of the UV-Vis detector was set at 254nm [8].

Instrumentation

An isocratic HPLC (Shimadzu HPLC Class VP series) with two LC- 0 AT VP pumps (Shimadzu), a variable wave length programmable photo diode array detector SPD-M10A VP (Shimadzu), a CTO- 10AS VP column oven (Shimadzu), a SCL-10A VP system controller (Shimadzu), a reverse phase Luna 5µl C18 (2) and Phenomenex column (250 mm X 4.6mm) were used. The mobile phase components Methanol:water (45:55) were filtered through a 0.2µ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 260-270kgf/cm^2. The column temperature was maintained at 27°C. 20µl of the respective sample and was injected by using a Rheodyne syringe (Model 7202, Hamilton).

FTIR analysis

FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum [9].

RESULTS AND DISCUSSION

UV-Visible spectrum analysis

The UV-Visible spectrum of the methanol extract of *Oxystelma esculentum* R.Br. was selected at the wavelength of 200nm to 900nm due to the sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 250, 300, 350, 400, 450, 502, 534, 606, 664 and 700 with the absorption 1.699, 3.568, 3.505, 3.374, 0.867, 0.509, 0.423, 0.423, 1.001 and 0.018 respectively (Table 1 and Figure 2).

HPLC analysis

The qualitative HPLC fingerprint profile of the methanol extract of *Oxystelma esculentum* R.Br. was selected at a wavelength of 660nm due to the sharpness of the peaks and proper baseline. The methanol extract prepared by hot extraction was subjected to HPLC for the separation and identification of constituents present in the *Oxystelma esculentum* R.Br., Four compounds were separated at different retention times of 1.867min, 2.063min, 2.797min and 3.000min respectively. The profile displayed three prominent peaks at the retention times of 1.687min, 2.063min and 2.797min, followed by only one moderate peaks were also observed at the retention times of 3.000min (Figure 3).

FTIR ANALYSIS

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infra red radiation. The crude methanol powder of *Oxystelma esculentum* R.Br. was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The results of FTIR analysis of methanol extract showed different peaks at 652.86, 680.83, 1039.56, 1071.38, 1215.07, 1372.26, 1453.26, 1511.12, 1608.52, 1732.92, 2854.45, 2932.56 and 3442.70. It was confirmed the presence of functional groups such as sulphonic acid (S-O stretching), sulphates, cycloalkanes (skeletal vibration), phenyl, primary amines (C-N stretching), methane (CH stretching), alkanes (asymmetry stretching), aromatic (C=C skeletal stretching), nitrate esters (NO_2 asymmetry stretching), 2-halo, alkanes and lactams (NH stretching) group respectively (Figure 4).

Table 1. UV-Visible spectrum of methanol extract of *Oxystelma esculentum* R.Br.

Nm	250	300	350	400	450	502	534	606	664	700
Abs	1.699	3.568	3.505	3.374	0.867	0.509	0.423	0.423	1.001	0.018

Figure 1. Natural habit of *Oxystelma esculentum* R.Br.

Figure 2. UV-Visible spectrum of methanol extract of *Oxystelma esculentum* R.Br.

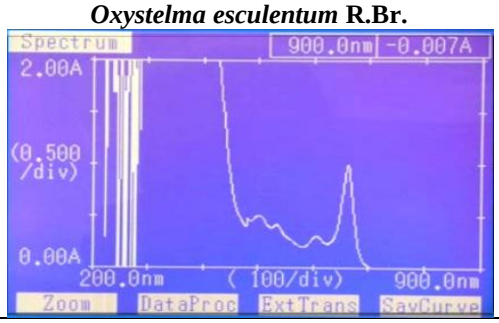


Figure 3. HPLC analysis of methanol extract of *Oxystelma esculentum* R.Br.

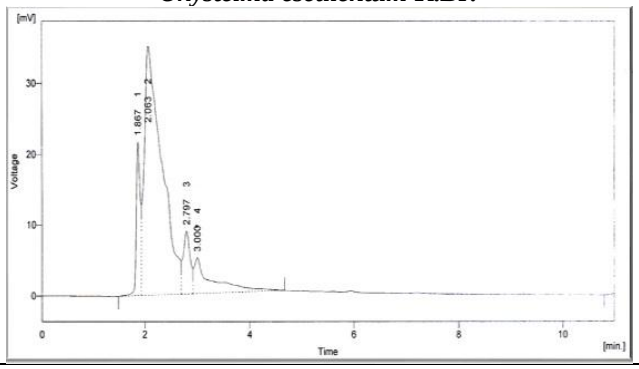
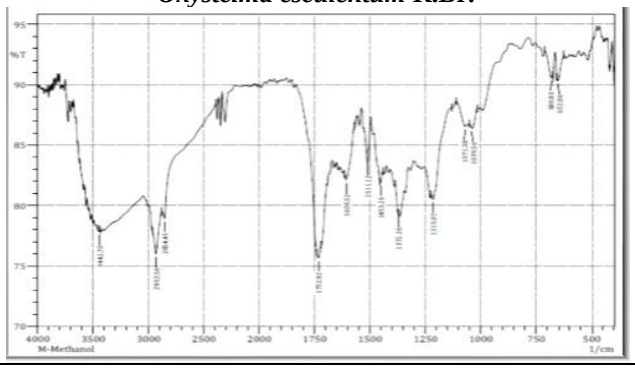


Figure 4. FT-IR spectrum of methanol extract of *Oxystelma esculentum* R.Br.



CONCLUSION

From the present study, it was concluded that UV-Visible spectrum showed the compounds separated at the nm of 250, 300, 350, 400, 450, 502, 534, 606, 664 and 700 with the absorption 1.699, 3.568, 3.505, 3.374, 0.867, 0.509, 0.423, 0.423, 1.001 and 0.018 respectively. The qualitative HPLC fingerprint profile displayed seven compounds at different retention times. The profile displayed four compounds at different retention times of 1.867min, 2.063min, 2.797min and 3.000min. The profile displayed three prominent peaks at the retention times of 1.687min, 2.063min and 2.797min, followed by only one moderate peak at the retention time of 3.000min. The result of FTIR analysis was found the presence of functional groups such as sulphonic acid, sulphates, cycloalkanes, phenyl, primary amines, methane, alkanes, aromatic, nitrate esters, 2-halo, alkanes and lactans.

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