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SCREENING OF PHYTOCHEMICALS OF CHLOROFORM EXTRACT OF *OXYSTELMA ESCULENTUM* R.BR.

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ABSTRACT

The present study was undertaken to screen the phytochemicals present in the chloroform extract of *Oxystelma esculentum* R.Br. The chloroform 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 400, 450, 500, 536, 606, 664, 700, 750, 800 and 882 with the absorption 3.675, 2.237, 1.734, 1.606, 1.473, 1.931, 1.190, 1.125, 1.071 and 1.010 respectively. The qualitative HPLC fingerprint profile showed ten compounds at different retention times. The profile displayed six prominent peaks at the retention times of 1.650min, 2.113min, 2.347min, 2.433min, 2.800min and 3.013min followed by four moderate peaks were noticed at the retention time of 5.217min, 5.957min, 7.190min and 8.640min. 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 showed the presence of functional groups such as di-sulphate, sulphades, phenyl nucleus, alcohols, hydroxyl group, nitrates, isopropyl, aromatic ring, α-helo and alkanes.

Keywords: *Oxystelma esculentum*, Phytochemicals, Chloroform extract, UV-Visible, HPLC, FTIR.

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INTRODUCTION

Naturally, there are more than thousands of known and many unknown phytochemicals present in the plants. It is well known that plants synthesize phytochemicals to protect themselves from these surroundings, but recent researches demonstrate that many phytochemicals can also protect human against various diseases. Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases. Many of these benefits suggest a possible role for phytochemicals in the prevention and treatment of disease, because of this property. Many researchers have been performed to reveal the beneficial health effects of phytochemicals [1].

An assessment of the previous trends and impact of research into the phytochemistry on medicinal plants of the world is quite desirable before considering recent trends. After centuries of empirical use of herbal preparation, the first isolation of active principles alkaloids such as morphine, strychnine, quinine etc. in the early 19th century marked a new era in the use of medicinal plants and the beginning of modern medicinal plants research. Emphasis shifted away from plant derived drugs with the tremendous development of synthetic pharmaceutical chemistry and microbial fermentation

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after 1945. Plant metabolites were mainly investigated from a phytochemical and chemotaxonomic viewpoint during this period.

Over the last decade, however, interest in drugs of plant and probably animal origin has grown steadily. Utilization of medicinal plants has almost doubled in Western Europe during that period. Ecological awareness, the efficacy of a good number of phytopharmaceutical preparations, such as ginkgo, garlic or valerian and increased interest of major pharmaceutical companies in higher medicinal plants as sources for new lead structures has been the main reasons for this renewal of interest. With the development of chemical science and pharmacognosy physicians began to extract chemical products from medicinal plants [2]. In this background, the present investigation was carried out to screen the phytochemicals present in the chloroform extract of *Oxystelma esculentum* R.Br.

MATERIALS AND METHODS

Collection of plant materials

The plant material selected for the present study is *Oxystelma esculentum* R.Br. 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 chloroform extract, 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 chloroform for 8h separately [3].

UV-Vis spectral analysis

The chloroform crude extract containing the bioactive compound was analyzed UV-Vis spectroscopically for further confirmation. The chloroform crude extract of *Oxystelma esculentum* R.Br. was scanned in a wavelength ranging from 310-900nm using a Shimazdu spectrophotometer and characteristic peaks were identified [4].

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 [5].

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 carried out 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 [6].

RESULTS AND DISCUSSION

UV-Visible spectrum analysis

The UV-Visible spectrum of the chloroform 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 400, 450, 500, 536, 606, 664, 700, 750, 800 and 882 with the absorption 3.675, 2.237, 1.734, 1.606, 1.473, 1.931, 1.190, 1.125, 1.071 and 1.010 respectively (Table 1 and Figure 1).

HPLC analysis

The qualitative HPLC fingerprint profile of the chloroform extract of *Oxystelma esculentum* R.Br. was selected at a wavelength of 660nm due to the sharpness of the peaks and proper baseline. The chloroform extract prepared by hot extraction was subjected to HPLC for the separation and identification of constituents present in the *Oxystelma esculentum* R.Br. Ten compounds were separated at different retention times of 1.650min, 2.113min, 2.347min, 2.433min, 2.800min, 3.013min, 5.217min, 5.957min, 7.190min and 8.640min respectively. The profile displayed six prominent peaks at the retention times of 1.650min, 2.113min, 2.347min, 2.433min, 2.800min and 3.013min followed by four moderate peaks were also observed at the retention times of 5.217min, 5.957min, 7.190min and 8.640min (Figure 2).

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 chloroform extract 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 chloroform extract showed different peaks at 516.89, 652.86, 675.04, 1058.85, 1230.50, 1366.57, 1624.92, 1738.71, 2917.13 and 3421.48. It was confirmed the presence of functional groups such as di-sulphate, sulphades, phenyl nucleus, alcohols (C-O stretching) hydroxyl group, nitrates, isopropyl (CH_3 symmetry), aromatic ring (C=C stretching), α-helo, alkanes (asymmetry stretching) and intermolecular hydrogen bonded OH (Figure 3).

Plants are considered to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected [7-8]. Extracts of plants were reported to exhibit antibacterial activity [9]. Several workers have reported that the plant extracts exhibit inhibitory activity against a number of gram positive and gram negative bacterial pathogens. A number of plants have been studied for their antibacterial activity both in India and abroad [10-12]. The presence of the secondary metabolites of *Oxystelma esculentum* R.Br. helps to study the various bioactivities of this plant.

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

Nm	400	450	500	536	606	664	700	750	800	882
Abs	3.675	2.237	1.734	1.606	1.473	1.931	1.190	1.125	1.071	1.010

Figure 1. UV-Visible spectrum of chloroform extract of *Oxystelma esculentum* R.Br.

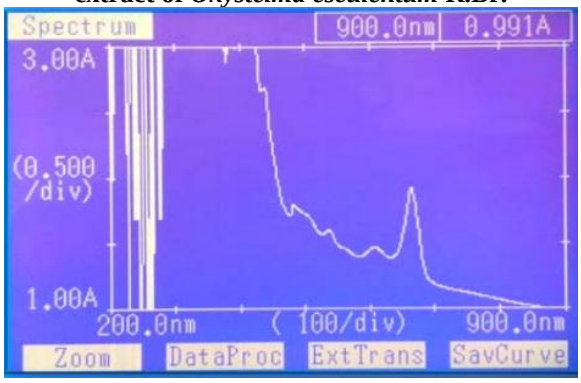


Figure 2. HPLC analysis of chloroform extract of *Oxystelma esculentum* R.Br

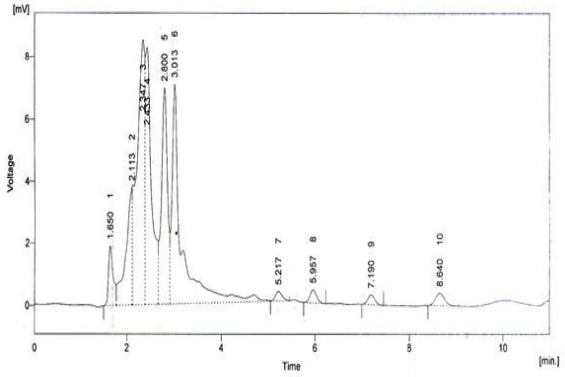
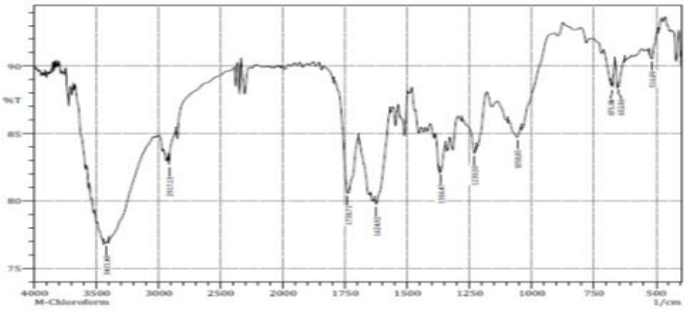


Figure 3. FT-IR spectrum of chloroform extract of *Oxystelma esculentum* R.Br.



CONCLUSION

From the present results, it was observed that UV-Visible spectrum showed the compounds separated at the nm of 400, 450, 500, 536, 606, 664, 700, 750, 800 and 882 with the absorption 3.675, 2.237, 1.734, 1.606, 1.473, 1.931, 1.190, 1.125, 1.071 and 1.010 respectively. The qualitative HPLC fingerprint profile showed ten compounds at different retention times. The profile displayed six prominent peaks at the retention times of 1.650min, 2.113min, 2.347min, 2.433min, 2.800min and 3.013min followed by four moderate peaks were noticed at the retention time of 5.217min, 5.957min, 7.190min and 8.640min. 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 showed the presence of functional groups such as di-sulphate, sulphades, phenyl nucleus, alcohols, hydroxyl group, nitrates, isopropyl, aromatic ring, α-helo and alkanes.

REFERENCES

1. Narasinga. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. *Asia Pacific Journal of Clinical Nutrition*, 12(1), 2003, 9-22.
2. Hamburger M and Hostettmann K. Bioactivity in plants: The link between phytochemistry and medicine. *Phytochemistry*, 30, 1991, 3864-3874.
3. John Peter Paul J and Muthu Sheeba M. Atomic Absorption Spectroscopic determination and comparison of some mineral elements in *Ulva rigida* C.Ag. from Hare Island, Thoothukudi, Tamil Nadu, India. *World Journal of Pharmaceutical Research*, 3(4), 2014, 785-795.
4. John Peter Paul J. Phytochemical studies on *Turbinaria ornata* (Turner) J.Ag. *American Journal of PharmTech Research*, 2(6), 2012, 582-589.
5. John Peter Paul J and Yuvaraj P. Phytochemical analysis of *Padina distromatica* Hauck. *Indo American Journal of Pharmaceutical Research*, 3(7), 2013, 5290-5297.
6. John Peter Paul J and Shri Devi SDK. Phytochemical Screening of *Padina tetrastromatica* Hauck. *American Journal of PharmTech Research*, 3(5), 2013, 214-222
7. Lindequist U and Schweder T. Marine Biotechnology. In: Rehm HJ, Reed G. (Eds.), *Biotechnology*, 10, 2001, 441-484.
8. Newman DJ, Cragg GM and Snader KM. Natural products as source of new drugs over the period 1981-2002. *J. Nat. Prod*, 66, 2003, 1022-1037.
9. Mahasneh I, Jamal M, Kashashneh M and Zibdeh M. Antibiotic activity of marine algae against multiantibiotic resistant bacteria. *Microbios*, 83, 1995, 23-26.
10. Burkholder PR, Burkholder LM and Almodovar LR. Antibiotic activity of some marine algae of Puerto Rico. *Bot. Mar*, 2, 1960, 149-156.
11. Allen MB and Dawson NY. Production of antibacterial substances by benthic tropical marine algae. *J. Bacteriol*, 79, 1960, 459-460.
12. Nunez G and Sanabria LMS. Effects of products by extracts of macroscopic marine algae on bacterial growth. *Acta. Biol. Venez*, 9, 1975, 121-134.

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