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FT-IR and GC-MS analysis of Evolvulus alsenoides for antidiabetic and anticancer drug identification

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ABSTRACT: The study was aimed to investigate the biochemical constituents of evolvulus alsenoides by methanolic extraction. Phytochemical screening was done with standard NIST procedures. GC-MS analysis revealed the major phytochemical constituents are 3-tert-Butyl-4 hydroxy anisole, Asarone, 4-hydroxy-2 methoxy cinnamaldehyde, 2-allyl,4-dimethoxy-3 methyl-benzene, benzenamine,N-[2(3,4-dimethoxy benzylamine)-1-(2-methyl-24-tetrazol-5),stigmata5,22-diene,3-methozy-(3-beta22E).The biological studies are based on pass online data bases.
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Keywords: Evolvulus alsenoides; Soxhlet extraction; FTIR; GC-MS; Anti-diabetic and anti cancer

1 Introduction

Evolvulus alsenoides belonging to the family convolvulaceae. It is a perennial herb used in Ayurvedic medicine [1]. Its application in the area of neuro degenerative diseases. The functional groups are alkaloids, flavonoids and pheolics were identified. Recent pharmacological studies on leaves and whole plant of evolvulus alsenoides having anti ulcer [2]. Using GCMS techniques bioactive components were analyzed. Isolation of compound exhibit antidiabetic activities [3-7].

2. Experimental

2.1. Collection and processing of plant material

The leaves of the plant Evolvulus alsenoides were collected from the natural habitats of Kanchipuram district, Tamil Nadu, India. The samples were washed with sterile distilled water. The roots were cut, shade dried, ground into fine powder and stored in air tight polythene bags until use.

2.2. Plant sample extraction:

Leaves were cleaned with tap water and again cleaned with distilled water and then allowed to shade dry and pulverized to powder using a mechanical grinder. 250grams of powdered material is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the soxhlet extractor. 2.5L of Methanol is taken into a distillation flask and the soxhlet is then equipped with a condenser. Methanol is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber house using the thimble of solid. The condenser ensures that any solvent vapour cools and drips backdown into the chamber housing in the solid material. The chamber containing the solid material is slowly filled with warm solvent. When the soxhlet chamber is almost full the chamber is automatically emptied by a siphon side arm with the solvent running backdown to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle may allowed to repeat many times over 8 hours during each cycle a portion of the nonvolatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. After extraction the solvent is removed by rotary evaporator and extracts were collected for analyzing GC-MS, FTIR-ATR.

2.3. FTIR -Spectroscopic analysis

The extracts were examined under FTIR spectrophotometer analysis; the extracts were centrifuged

at 3000 rpm for 10 min and filtered through Whatmann No. 1filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-1100 nm using Perkin Elmer Spectrophotometer/ATR and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer / ATR system, which was used to detect the characteristic peaks and their functional groups. The peak values of FTIR were recorded in each and every analysis was repeated twice for the spectrum confirmation [8].

2.4. GC-MS analysis

GC-MS analysis of leaves extracts was performed at VIT university, Vellore, Tamilnadu. 2µl of the methanolic extract of evolvulus alsenoides was employed for GC-MS analysis. The clarus 680 GC used in the analysis employed a fused capillary column packed with Elite-5MS [5%diphenyl,95%dimethyl polysiloxane] 30 mm × 0.25 mm ID × 250µm df] and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The 2µl sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.4.2 software. During the 32ndminute GC extraction process, the oven was maintained at a temperature of 110°C with 2 minutes holding. The injector temperature was set at 250°C (mass analyser). The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200°C; Source temperature: 200°C). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 50 to 600 Da. The MS detection was completed in 32minutes [9].

2.5. Identification of compounds

Compounds were identified in area by bit by bit through NIST ver 2.0 year 2008.

2.6. Preliminary phytochemical screening

Freshly prepared extracts were allowed to a standard phytochemical analysis to ensure major chemical components by standard descriptions.

2.7. PASS online

PASS (Prediction of Activity Spectra for Substances) estimates probabilities of belonging to the classes of 'actives' (Pa) and 'inactives' (Pi) for over 4000 biological activities from structure- activity relationships' analysis for the training set, including more than 300,000 biologically active compounds. The machine learning method implemented in PASS is based on the Bayesian algorithm and Multilevel Neighbourhoods of Atoms (MNA) descriptors. There are three options to input the structural formula of the studied compound to this web service: upload SMILES or MOL files .As an output, the user obtains the list of probable activities with the estimated Pa and Pi values, which is arranged in the descending order of Pa to Pi. PASS validation by leave-one-out and 20-fold cross-validation gave an average accuracy of prediction of about 95%. To avoid the overfitting, all information about the substance from PASS training set, which is equivalent to the substance under prediction, is excluded from SAR Base during the training procedures, which are equivalent to the substance under prediction. The chemical structures are considered equivalent in PASS if their molecular structures have the same set of MNA descriptors. Thus, if PASS Online input contains the structure of the drug, which is included in the PASS training set, during the prediction all information about this drug is excluded and prediction is performed based on the remaining part of the PASS knowledge base.

3. Results and Discussion

Preliminary phytochemical investigation confirmed presence of alkaloids,flavonoids and phenolics which establishes richness o plant in secondary metabolites.The results findouts,what are all the compounds from our work patterns. It will found out through chromatogram.Recorded value from the methanol extraction proves for the responsible of antidiabetic and anticancer activity.

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in Table 1. The solvent had its respective functional group like aliphatic amines, alkyl halides, esters, ketones alkanes, Alkenes etc. Hence, the crude extracts subjected to FTIR analysis is used for the identification of chemical constituents present in evolvulus alsenoides is labeled in figure 1. In FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition.

GC-MS analysis was done using the organic solvent methanol and it shows the presence of 6 different chemical compounds present in evolvulus alsenoides vide (Table 2 & Figure 2).

Table 1 FTIR peak values and functional groups of methanol extracts of evolvulus alsenoides.

S.No	Peak values	Functional group
1	2924	Alkanes
2	2854	Alkanes
3	1736	Ester.Ketones
4	1455	Alkanes
5	1377	Sulphoxides
6	1165	Aliphatic amines
7	981	Alkenes
8	889	Aromatics
9	722	Alkanes
10	591	Alkyl Halides

Table 2 Phytocomponents identified in methanolic leaf extract of evolvulus alsenoides using GC-MS

S.No	*RT*	*Name of compound*	Molecular formula	MW	Peak area	Biological activity
1	3.78	3-tert-Butyl-4 hydroxy anisole	$C_{11}H_{16}0_2$	180	6.03	Anti diabetic
2	4.44	Asarone	$C_{12}H_{16}O_3$	208	2.00	Anti malarial
3	6.35	4-hydroxy-2 Methoxy Cinnamal dehyde	$C_{10}H_{10}O_3$	178	6.02	Anti fungal
4	10.88	2-allyl,4-dimethoxy-3 methyl-benzene	$C_{12}H_{16}O_2$	192	5.14	Anti neuro
5	24.16	benzenamine,N-[2(3,4-dimethoxy benzylamine)-1-(2-methyl-24-tetrazol-5	$C_{16}H_{16}N_2O_4$	302	5.37	Anti oxidant
6	24.37	stigmata5,22-diene,3-methozy-(3-beta22E	$C_{30}H_{50}O$	426	1.11	Anti cancer

The sample was extracted with methanol because of the effect of antidiabetic and anticancer activity. 6 components from the chromatogram with retention time 3.78 & 24.37 respectively [11, 12].

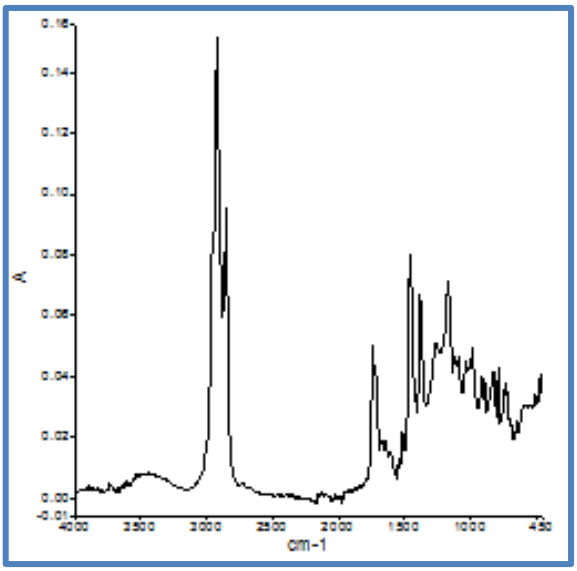

Figure 1 FTIR spectrum of methanol extracts of evolvulus alsenoides.

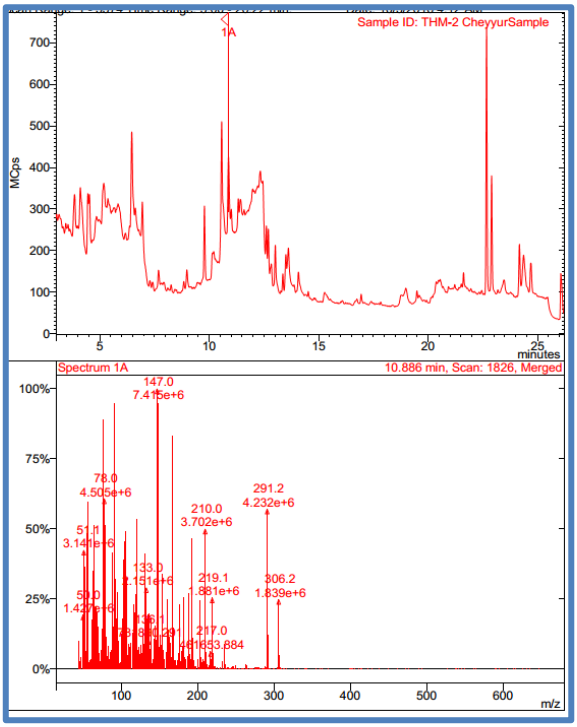

Figure 2 GC-MS analysis of methanol extract of evolvulus alsenoides.

4 Conclusions

The present investigation supports the traditional ethnomedicinal claims of evolvulus alsenoides for the treatment of diverse infections. Identified phyto components using GC-MS can be used as a commercial device for the identification of pass online biological activity predicts. It opens the way to evolve different treatment regimens used this extract. Further research is necessary to need to treat this plant material is an effective anti-diabetic and anticancer agent.

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Competing Interests:

The authors declare that they have no competing interests.

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