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Isolation and characterization of Swertiamarin from aerial parts of *Enicostemma littorale* blume

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Abstract: *Enicostemma littorale* Blume (Fam. Gentianaceae) is commonly known as Nagajihva, is used in traditional system of medicine since long time. Many compounds have been reported from the various parts of the plant. In the present study, n-butanol soluble extract of the aerial parts of the plant was subjected to column chromatography to isolate and purify the phytoconstituents which can be termed as marker. One compound was isolated and characterized by NMR (Nuclear Magnetic Resonance), IR (Infra-red Spectroscopy), and Mass spectroscopy as Swertiamarin. Novel HPLC (High Performance Liquid Chromatography) methods were developed for assessment of purity, for standardization and for estimation of the compound. Simplicity of isolation and HPLC analysis of the compound suggests that the compound may be termed as markers for the standardization of the extracts and preparations containing *Enicostemma littorale*.

Keywords: *Enicostemma littorale* Blume, Phytoconstituents, Characterization, Markers, HPLC

Introduction

Herbal drugs have played a vital role in curing diseases throughout history of mankind. Now medicine plants recognized globally as important resources for all major system of medicines, health care, neutraceuticals, phytochemicals and cosmetics.

Since 1980, the World Health Organization has been encouraging countries to identify and exploit traditional medicine and phytotherapy [1].

Mass screening of plants in the search for new drugs is vastly expensive and inefficient but it would be cheaper and more productive [2]. Fortunately, the interest and concern to discover novel compounds from natural origins are ever on the increase. We should be aware of the fact that some global pharmaceutical companies have already engaged themselves in the intense and systematic research so as to develop new medicines from nature [3,4]. The process of structural determination involves accumulating data from numerous sources, each of which gives some structural information, and the assimilation of these data into a chemical structure that uniquely fits all the available structural information. A wide range of spectroscopic instrumentation, such as UV (Ultra Violet Spectroscopy), IR, and visible absorption spectroscopies, NMR spectroscopy and Mass spectroscopy forms the backbone of the modern structural analysis [5].

Marker compounds are the constituents believed to be peculiar to the medicinal and aromatic plant, but which may not be the bioactive principles in the plant [6]. Several medicinal plants have been described to be beneficial for diabetic ailments in "Atharva Veda" an ancient treatise from which Ayurveda, the Indian system of Medicine owes its origin [7]. The present work aims to the standardization of phytochemical constituents present in methanol, ethyl acetate and chloroform extract of aerial parts of *Enicostemma littorale* blume.

Materials and Methods

Collection of plant material

Authenticated dried aerial parts of *Enicostemma littorale* was collected from Botanical Garden, Tirupathi subjected to extraction with different solvents.

Preparation of extracts of plant materials

The plant materials were dried in the laboratory under shade and were pulverized to a coarse powdered and grinded material was used for the extraction process. These powdered material were subjected to soxhlet extraction using methanol, ethyl acetate and

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chloroform. Each 5 g of dried powder was filled separately in the thimble and extracted successively with 60 mL of solvents using a soxhlet extractor for 3 hours. After solvent evaporation, each of these solvent extract was weighed and preserved in room temperature until further use.

HPLC analysis

Samples were subjected to HPLC analysis. Homogeneity of plant extracts were assessed by High Performance Liquid Chromatography. 1 mg of each sample was dissolved in 1 mL of solvent. If there is some undissolved material, then the sample was filtered through a 0.22 µm filter. 10 µL of the prepared sample was injected and linear gradient from 0% to 100% solvent B was used over 30 min to elute the sample. Typical chromatograms of the samples were analysed.

IR analysis

Infra-red spectra of the compounds were recorded using IR spectrophotometer. Small drop of the compound was placed on one of the KBr (Potassium Bromide) plates. The second plate was placed on top and a quarter turn was made to obtain a nice even film. Then plates were placed into the sample holder and spectrum was run.

NMR spectrum

NMR spectrum of the compounds were recorded using a Nuclear magnetic resonance spectroscopy.

Results

Physicochemical paramaters

Table 1: Determination of moisture values.

S.No	Parameter	% content
1	Loss of drying	6.68%
2	Moisture content	4.26%

Table 2: Determination of ash values. a Mean value of three readings.

S.No	Type of ash value	% w/w (Mean[a] ± SEM)
1	Total ash	4.05 ± 0.17
2	Acid insoluble ash	2.89 ± 0.02
3	Water soluble ash	3.90 ± 0.10
4	Sulphated ash	0.62 ± 0.21

Isolated compounds

The isolated compound from n-butanol extract labelled as EL-01. The Rf values for the above isolated compound was found to be (EL-01): 0.54. The yield of isolated compound was found to be 4.5 g and the physical properties of this compound was shown in the Tables 3 and 4.

Table 3: Physical properties of EL-01

Code	EL-01
Solubility	Methanol
State	Powder
Colour	Light yellow
Odour	Characteristic
Melting point	114^{0}C
Molecular weight	374.5

Table 4: Analytical HPLC result of EL-01.

Compound code	Time (min)	Concentration (%)	Area
EL-01	7.394	1.150	121062
	7.681	98.545	10373076
	8.747	0.305	32056

Spectroscopic analysis of the isolated compounds

The supporting IR values

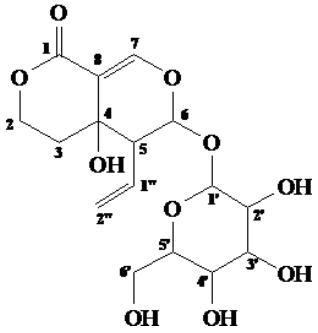
The IR spectram shows a band of 3450 cm^{-1} due to hydroxyl group. The band at 1750 cm^{-1} is due to the enone system conjugated with oxygen. A medium band at 1650 cm^{-1} shows the presence of double bonds in the molecule while the bands at 1390 to 860 cm^{-1} are due to COC and the terminal double band respectively.

^{1}H NMR spectrum (DMSO- 200 MHz)

The EL-01 ^{1}H NMR spectrum shows singles of hydrogen at C3 δ 1.73 as multiplets. The multiplets at δ 2.85 is due to the proton on C5, the glucosidic protons show multiplets between δ 2.93-3.49. The doublet at δ 4.47 is due to hydrogen at C1'. Multiplets at 4.12 and 4.30 are assigned to protons on C2. The olefinic protons on terminal methylene C2" appears as multiplets at 4.63 and 5.03 while multiplets due to the olefinic protons on C1" appears at δ 5.14. The doublet at δ 5.15 is assigned to the proton on C6 attached to oxygen's. The olefinic proton on C7 shows a singlet at δ 7.5 (Tables 5).

LC-MASS data

m/z 771.27 (100%); 397.50 (32%).



Molecular Formula: $C_{16}H_{22}O_{10}$

Table 5: Supporting ^{1}H- NMR values for EL-01.

^{1}H	Lit-1 (ICMR) DMSO-D_6	Lit-2 300MHz CD_3OD	NRPL 200MHz DMSO
3α	1.4 (1H, m)		
3β	**1.75** (1H, m)	1.74 (ddd, J-13.5,4.1,12.0 Hz)	–
5	2.91 (1H, m)	**1.84** (ddd, J-13.5,1.7,2.2Hz) 2.93 (dd, J-1.8,9.4Hz)	1.73 (2H, m)
Glucosidal protons	3.20-3.81 (m)	3.16-3.50 (m) 3.70 (dd, J-12.0,2.1)	2.85 (1H, m) 2.93-3.49 (m)
6' α	–	3.90 (dd,12.5,2.2,11.4)	3.73 (1H, dd, J-6.68,6.5)
2α	3.91 (1H, m)	4.35 (ddd, J-4.2,1.7,11.4)	4.12 (1H, m)
2β	4.30 (1H, m)	4.64 (d, J-7.8 Hz)	4.30 (1H, m,)
1'		–	4.47 (1H, J-7.72 Hz) 4.63 (1H, m, J-4.84 Hz)
2''α	4.15 (1H, d, J-8Hz)	–	5.03 (1H, m)
2''β	4.85 (1H, m) 4.95 (1H, m, J-5 Hz)	–	5.14 (1H, m)
1''	5.10 (1H, m)	5.72 (d, J-1.7Hz)	5.29 (1H)
6	5.15 (1H, d, J-2.4Hz)	7.62 (d,J-2.4Hz)	7.51 (1H, s)
7	7.26 (1H,s)		

[4aR-(4aa,5b,6a)]-5-Ethenyl-6-(b-D-glucopyranosyloxy)-4,4a,5,6-tetrahydro-4a-hydroxy-1H,3H-pyrano[3,4-c] pyran-1-one.

Estimation of EL-01 in different extracts of *E.littorale* by HPLC

Peaks were observed in the similar retention time corresponding to the standards confirmed the presence of the isolated compound in the various extracts; MeOH extract, ethyl acetate fraction extract, n-butanol fraction extract, water fraction extract, 80% MeOH in water extract, 100% ethyl acetate extract, chloroform extract and successive 75% MeOH in water extract.

The Assay (% w/w) of the isolated compound in the MeOH extract, ethyl acetate fraction extract, n-butanol fraction extract, water fraction extract, 80% MeOH in water extract, 100% ethyl acetate extract, chloroform extract and successive 75% MeOH in water extracts were determined, shown in the Tables 6.

Table 6: Standardisation values of different extracts.

Sample name	Swertiamarin (EL-01)
Methanol extract	6.54
Ethyl acetate fraction extract	0.04
n-butanol fraction extract	2.60
Water fraction extract	0.37
80% MeOH in water extract	4.35
100% Ethyl acetate extract	0.89
Chloroform extract	0.28
Successive 75% MeOH in water Extract	2.18

Discussion

Standardization carried out by means of chemical, physical or analytical methods, may not correlate with the biological or pharmacological activities claimed, they should be standardized uniformly [8]. For standardization of natural product drugs, single chemical entities; "Marker Compounds" may be used as potency standards in High Performance Liquid Chromatography (HPLC) analysis [9].

The aerial parts of *Enicostemma littorale* blume belonging to the family Gentianaceae, was selected for the isolation of phyto constituents. The Methanol extract was dissolved in water and partitioned with Ethyl acetate, n-butanol and n-butanol subjected to column chromatography. The isolated, purified compound was characterized by determining their physical properties like solubility, melting point and by subjecting them to NMR (13C and 1H), Mass spectroscopy and IR spectroscopy. The name of the isolated compound EL-01 was confirmed as Swertiamarin. In this work, we finally conclude that

the column chromatography method employed for isolation of EL-01was found to be suitable for commercial scale.

Conclusion

The isolated compound was used to standardize the MeOH extract, Ethyl acetate fraction extract, n-butanol fraction extract, water fraction extract, 80% MeOH in water extract, 100% Ethyl acetate extract, Chloroform extract and successive 75% MeOH in water extracts of the aerial parts of *Enicostemma littorale* blume by novel HPLC method. The name of the isolated compound EL-01 was confirmed as Swertiamarin. In this work, we finally conclude that the column chromatography method employed for isolation of EL-01was found to be suitable for commercial scale. Further investigations are required to understand the mechanisms of action underlying the effects of the extract and their active compounds.

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