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Phytochemical analysis of stem extract of *Cynanchum viminalis* L. by HRLC-MS analysis

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Abstract

Cynanchum viminalis L. a species with variety of phytochemical compounds which has potential to be used as medicine. Present investigation was undertaken in order to analyse bioactive constituents in stem of *C. viminalis* by using High Resolution Liquid Chromatography- Mass Spectrometry (HRLC- MS).

The present study revealed that the presence of phytochemical compounds like- Ancistrocladine, Piperolein B, 1, 1'-Bis (2-hydroxy-3- methylcarbazole), Retinyl beta-glucuronide, Chrysophanol 1-triglucoside, Gentamicin A, Cerberin, Nystatin A1, 2, 3-Dihydrowithanolide E, Irinotecan, Grossamide, Kaempferol 3-O-beta-D-galactoside, Ellagic acid, Gingerglycolipid A, Agavasonin C and Fistuloside C which belongs to various groups.

Keywords: *Cynanchum viminalis*, HRLC- MS, Withanolide, saponins, polyphenolic

Introduction

Sarcostemma acidum (Roxb.) Voigt is a synonym of *Cynanchum viminalis* L. It is a perennial shrub belongs to the family Asclepiadaceae commonly known as milkweed family [1]. It has green, cylindrical branches and milky white latex flowers. The shrub is distributed in India, Sri Lanka, Pakistan, and Europe [2]. Gamopetalous corolla, staminal column, corona and peltate style apex, formation of pollinia and follicular fruits and flattened seeds with silky comas are characteristics of the *S. acidum* [3]. *C. viminalis* a species with variety of phytochemical compounds has potential to be used as medicine. The traditional knowledge of utilizing plants in medicine and food related to the family Asclepiadaceae for diabetes, headache, kidney stone, swelling, fever, wounds, snake bites and skin problems [4]. *S. acidum* has been used in folklore to treat a variety of mental health issues. It has been covered in ancient Indian literature "Shrusrutha Samhitha" [5].

The current research focuses on its phytochemical constituents by using High Resolution Liquid Chromatography- Mass Spectrometry (HRLC- MS) technique.

Materials and Methods

The healthy stem of *C. viminalis* was collected from the Gogababa Hill, University Campus, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajanagar, Maharashtra 431004 on 19 October 2023. The herbarium specimen has been prepared [6] and voucher specimen was submitted to BAMU Herbarium having accession number-00928.

The stem of *C. viminalis* was washed, dried, and cut into small pieces. The dried stem was finely ground and powdered. About 25 gm of fine powder was used for the Soxhlet's extraction. Extraction will be done in various solvents like ethanol, petroleum ether, and chloroform. The crude extract of stem was obtained. This extract was found to be soluble completely in Dimethyl Sulfoxide (DMSO) Solution. The extract was screened for qualitative analysis which was carried by instrument QTOF, Version: Q-TOF B.05.01 (B5125.3) to obtained phytochemical constituents by using HRLC-MS technique at Sophisticated Analytical Instrument Facility, IIT, Bombay.

Results and Discussions

Morphologically diagnostic characters of the *C. viminalis* is almost leafless, straggling, jointed shrub with many branches. Stem is cylindrical, green, pubescent in young stage and gradually turns smooth. Leaves are very minute, caducous, ad pressed and opposite. Gamopetalous corolla, staminal column, corona and peltate style, formation of pollinia and follicular fruits and flattened seeds with silky comas.

C. viminalis stem showed the presence of secondary metabolites in positive compatibility mode and negative compatibility mode which belong to various classes of Amide Alkaloids, Isoquinoline alkaloid, Flavouring agent, Oligosaccharides, Naphthopyrans, Aminoglycoside, Cardiac Glycoside, glycosides, Vitamin A, Vitamin D, Steroidal Saponins, Steroids, Polyene macrolide antibiotic, Anisoles, Phosphatidylethanolamines, Lignan amide, DNA topoisomerase I inhibitor. The biochemical major and minor compounds included are (+/-)-3-[(2-methyl-3furyl)thio]-2-butanone, Piperolein B, Chrysophanol 1-triglucoside, Ancistrocladine, Dihydrodeoxystreptomycin, Gentamicin A, Retinyl beta-glucuronide, Cerberin, Fistuloside C, Nystatin A1, 2,3-Dihydrowithanolide E, Irinotecan, Irinotecan, 1,1'-Bis (2-hydroxy-3- methylcarbazole, Chrysophanol 1-triglucoside, Kaempferol 3-O-beta-D-galactoside, Ellagic acid, Gingerglycolipid A, Agavasaaponin C.

Piperolein B has been reported to possess larvicidal, hepatoprotective and enzymatic inhibition activity. It has also been shown to be a Transient Receptor Potential agonist (TRP). The TRP agonists capsaicin and piperine have been shown to increase salivary flow when introduced into the oral cavity [7].

Chrysophanol triglucoside has the potential for diabetes research [8].

Gentamicin A is a constituent of the gentamicin complex, is a broad-spectrum anti-bacterial, anti-biotic [9].

Retinyl beta-glucuronide, a naturally occurring vitamin A metabolite, is a detoxification product and plays various roles in vitamin A functions. It is comparable to retinoic acid in treating acne without side effects and may be therapeutic for skin disorders and certain cancers [10].

Cerberin is a cardenolide glycoside that is the 2'-acetyl derivative of neriifolin. It has a role as an antineoplastic agent and a metabolite [11].

Cerberin natural products possess a significant role in anticancer therapy and many currently-used anticancer drugs are of natural origin. Cerberin a cardenolide, was found to potently inhibit cancer cell growth, colony formation and migration [12].

Fistuloside C were isolated from the edible parts of *Allium fistulosum* L. and their antimicrobial activity was evaluated with pathogenic- or food-spoilage microorganism based on disk-diffusion assay, minimal inhibitory concentration (MIC)

and minimal fungicidal concentration (MFC) determination. The fistuloside C showed a prominent antifungal activity with 3.1~6.2 ug/ml of MIC and MFC [13].

2,3-Dihydrowithanolide E is a withanolide found to have useful biological effects such as anticancer, radio sensitizing, antibacterial, adaptogenic, immunomodulating, antioxidant, hepatoprotective, antistress, anti-inflammatory, antiarthritic, and insect antifeedant activities [14].

Irinotecan also known as CPT-11, is a DNA topoisomerase I inhibitor used to treat solid tumours like colorectal, pancreatic, ovarian, and lung cancers. Its mechanism of action, adverse event profile, and key factors for healthcare team members are reviewed, providing essential information for patients receiving treatment [15, 16, 17].

Grossamide is a member of benzofurans, anti-inflammatory agents, antioxidant, anti-cancer and anti-hyperlipidaemic capacities *in vitro*, cell culture and *in vivo* studies. With strong potential to be utilized as protective agents against human chronic diseases, these compounds have attracted the interest of researchers [18]. 1,1'-Bis (2-hydroxy-3-methylcarbazole) alkaloids possess potent biological and pharmacological effects, including antioxidant, antidiabetic, anti-inflammatory, antitumor, and neuroprotective properties, making them effective in various applications [19].

Chrysophanol 1-triglucoside has been extensively studied for its potential therapeutic properties, including anti-inflammatory, anti-cancer, and anti-oxidant effects [20].

Kaempferol 3-O-beta-D-galactoside is a plant metabolite and antifungal agent, a monosaccharide derivative, antinociceptive and anti-inflammatory properties [21].

Ellagic acid (EA) is a natural phenolic constituent, recent *in vitro* and *in vivo* experiments have revealed that EA has been shown to exhibit anticarcinogenic effects, inhibiting tumour cell proliferation, promoting apoptosis, breaking DNA binding to carcinogens, and disrupting inflammation [22].

Gingerglycolipid A is a galactosyl glycerol derivative has antiemetic effect, it is effective against arthritic diseases, and against tumour growth, rheumatism, and migraine, anti-diabetic potential of ginger glycolipid A [23].

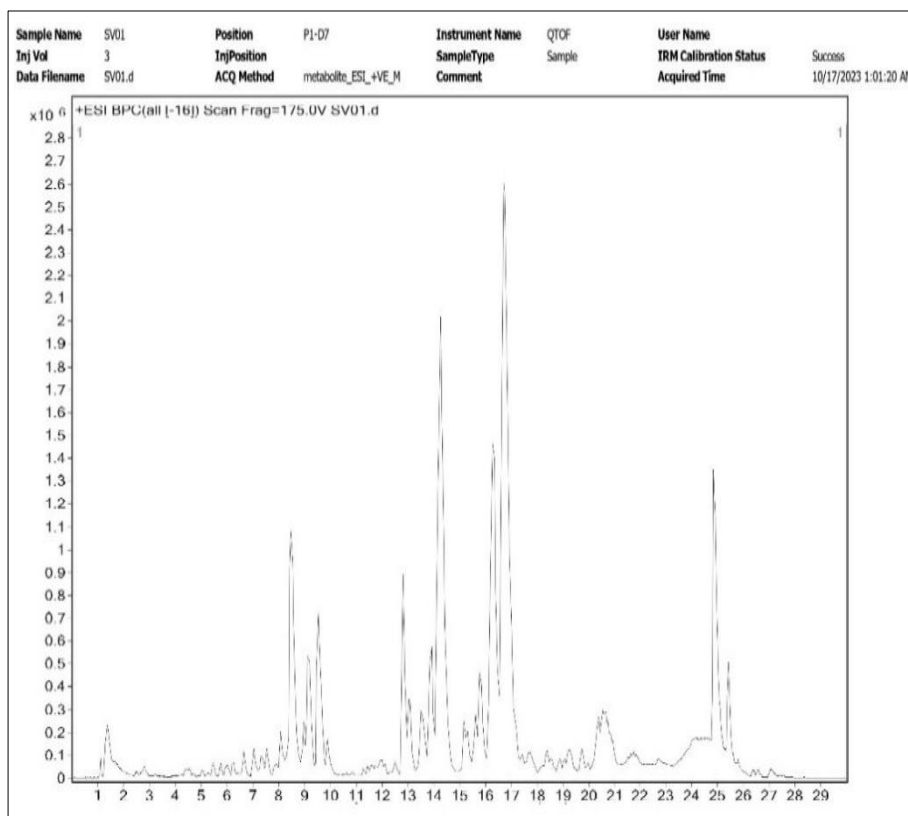
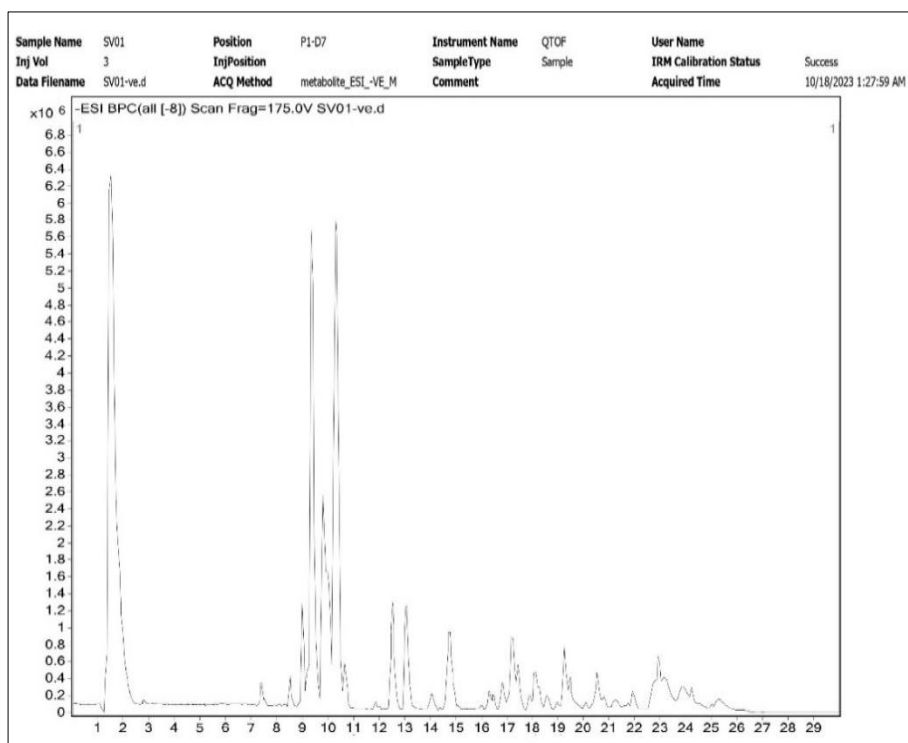
Agavasaaponin C is Saponin, the glycosides of steroids or triterpenes with numeral of pharmacological activities like anti-oxidant, immuno-stimulant, anti-inflammatory, anti-cancer, adjuvant, anti-microbial, hypo-cholesterol emic properties [24].

Table 1: Phytochemical compounds found in positive compatibility mode in stem of *C. viminalis*.

Sr. no.	Name	Formula	Group	Mass	RT	DB Diff (ppm)
01.	(+/-)-3-[(2-methyl-3furyl)thio]-2-butanone	C ₉ H ₁₂ O ₂ S	Flavoring agent	184.0559	1.117	-0.5
02.	Piperolein B	C ₂₁ H ₂₉ NO ₃	Amide Alkaloids	343.2169	6.666	-6.42
03.	Chrysophanol 1-triglucoside	C ₃₃ H ₄₀ O ₁₉	Oligosaccharides	740.2097	8.366	9.05
04.	Ancistrocladine	C ₂₅ H ₂₉ NO ₄	Isoquinoline alkaloid	407.211	11.835	-3.31
05.	14alpha-Hydroxypaxilline	C ₂₇ H ₃₃ NO ₅	Naphthopyrans	451.2368	11.926	-1.97
06.	Dihydrodeoxystreptomycin	C ₂₁ H ₄₁ N ₇ O ₁₁	Aminoglycoside	567.2843	12.821	3.64
07.	Gentamicin A	C ₁₈ H ₃₆ N ₄ O ₁₀	Aminoglycoside	468.2463	12.884	-6.71
08.	Retinyl beta-glucuronide	C ₂₆ H ₃₈ O ₇	Vitamin A	462.2591	13.559	5.69
09.	Cerberin	C ₃₂ H ₄₈ O ₉	Cardiac Glycoside	576.3266	13.607	5.63
10.	Fistuloside C	C ₄₅ H ₇₂ O ₁₉	Steroidal Saponins	916.4744	14.269	-8.28
11.	Nystatin A1	C ₄₇ H ₇₅ NO ₁₇	Polyenemacrolide antibiotic	925.4982	15.187	5.71
12.	Chinenoside VI	C ₄₄ H ₇₀ O ₁₉	Steroidal Saponins	902.4591	15.323	-8.83
13.	2,3-Dihydrowithanolide E	C ₂₈ H ₄₀ O ₇	Steroid lactones	488.2727	15.813	9.73
14.	Flavidulol C	C ₃₄ H ₄₂ O ₄	Anisoles	514.3086	17.769	-0.47
15.	Grossamide	C ₃₆ H ₃₆ N ₂ O ₈	Lignan amide (Benzofurans)	624.2515	24.085	-6.98
16.	Irinotecan	C ₃₃ H ₃₈ N ₄ O ₆	DNA topoisomerase I	586.2754	24.437	6.38

Table 3: Phytochemical compounds found in negative compatibility mode in stem of *C. viminalis*.

Sr. no.	Name	Formula	Group	Mass	RT	DB Diff (ppm)
01.	1,1'-Bis (2-hydroxy-3- methylcarbazole)	C ₂₆ H ₂₀ N ₂ O ₂	Alkaloids (Indoles and derivatives)	392.1537	7.567	-3.01
02.	Chrysophanol 1-triglucoside	C ₃₃ H ₄₀ O ₁₉	Anthraquinone Derivatives	740.2118	9.25	6.23
03.	Kaempferol 3-O-β-D-galactoside	C ₂₁ H ₂₀ O ₁₁	Monosaccharide Derivative	448.0967	10.068	8.7
04.	Ellagic acid	C ₁₄ H ₆ O ₈	Polyphenolic	302.0058	11.585	1.63
05.	Gingerglycolipid A	C ₃₃ H ₅₆ O ₁₄	Galactosylglycerl Derivative	676.3611	16.472	8.71
06.	Agavasaponin C	C ₄₅ H ₇₂ O ₁₉	Steroids	916.4759	17.226	-9.98

**Fig 1:** (HR)-LCMS Chromatogram for Positive Mode of *Cynanchum viminalis* stem**Fig 2:** (HR)-LCMS Chromatogram for Negative Mode of *Cynanchum viminalis* stem

Conclusion

The stem extract of *Cynanchum viminalis* L. revealed the presence of important phytochemical compounds like Chrysophanol 1-triglucoside, Gentamicin A, Retinyl beta-glucuronide, Fistuloside C, 2,3-Dihydrowithanolide E, Grossamide, 1, 1'-Bis (2-hydroxy-3-methylcarbazole, Kaempferol 3-O-beta-D-galactoside, Ellagic acid, Irinotecan, Gingerglycolipid A and Agavasaponin C which can be used for standardize herbal drugs.

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