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GC-MS screening of alklaoids of *Withania somnifera L. in vivo and in vitro*

KEYWORDS: *Withania somnifera*, GC-MS, Withasomnine, Anaferine

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ABSTRACT *In this study, roots and callus of Withania somnifera (family: Solanaceae) commonly called Ashwagandha were analyzed by GC-MS for their alkaloid content. GCMS analysis confirmed the presence of 17 alkaloids in the plant and maximum was present in roots (17) > callus (13). Alkaloids present are withasomnine:(4-phenyl-1,5-timethylene pyrazole); somniferine:[1-(3-4—dimethoxybenzyl)-6-7-dimethoxyiso-quinoline]; isopelletierine: 1(2-piperidinyl)2-propanone; anaferine: pyrazole[(1,3-bis,2R-piperidin-2-yl)propan-2-one)]; anahygrine: [1-amino-2(methoxymethyl) pyrrolidine]; tropine(3-end)-8-methyl-8 azabicyclo (3.2.1)octane-2-carboxylate; pseudotropine [Methyl (1R,2R,3S,5S)-3-(benzoyloxy)-8-methyl-8-azabicyclo (3.2.1)octane-2-carboxylate; Iron-pseudotropine: [Fe(1R,2R, 3S,5S)-3-(benzoyloxy)-8-methyl-8-azabicyclo(3.2.1)octane-2-carboxylate; withanine: 3α-glyoxy-tropane; choline; cuscohygrine; N-(3-acetylphenyl)-1-Adanantanecarbox-amide; 1-[(5-Nitro-2-furfurylidene) amino]; oxacyclohexadecane-2,13-dione,13-oxime; scopoletin: Ashwagandhine:2,4-imidazolidiendione1-[{(5-nitro-2-furanyl)methylene]amino}. The alkaloid withasomine(18.12% in roots and 10.72% in callus) and anaferine(10.72% in roots and 8.14% in callus) were the predominant alkaloids in W.somnifera .*

Introduction

Withania somnifera Dunal (Solanaceae), known in India as Ashwagandha or winter cherry, is one of the most valuable plants of the traditional Indian systems of medicines, is used in more than 100 formulations of Ayurveda, Unani and Sidha and is therapeutically equivalent to ginseng (Sangwan *et al.*, 2004).

Regarding secondary metabolites, Solanaceae plants are principally recognized as producers of tropane alkaloids – alkaloids that have also been isolated from *W. somnifera* – in fact, long before the isolation of withanolides from this plant (Khanna *et al.*, 1961). These alkaloids include N-methylpyrrolinium-derived nicotine alkaloids, tropine-derived true tropane alkaloids, and pseudotropine-derived nortropane alkaloids, also called calystegines (De Luca and St Pierre 2000, Griffin and Lin 2000; Drager, 2006).

Because, some of the species of the genus *Withania* exhibited pathological-physiological and biological-activities, their chemistry has been extensively studied, incidentally, very less attention has been paid to study the alkaloid pattern in *Withania* species, in particular, but very few reports have been published on the production of alkaloid in *Withania* species (Dhalla *et al.*, 1961; Schroter and Neumann, 1966; Parr *et al.*, 1990; Pati *et al.*, 2008; Soni *et al.*, 2010; Parvatham, 2011; Bhatt *et al.*, 2011; Sehgal *et al.*, 2012. The presence of high alkaloids levels in *W.somnifera* is responsible for the pharmacological properties and clinical symptoms. In view of this, no systematic work has been done on this *in vivo* and *in vitro alkaloid* content in the selected *W. somnifera.*

Material and Method

Plant material

The roots of *W. somnifera* were collected from the field of Jaipur region. The roots were washed with deionized water, shade dried till the weight become constant at room temperature and ground to fine powder. The species specimen was submitted in herbarium, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India and got the voucher specimen No. RUBL21148.

For callusing, nodal segments were cultured on MS medium supplemented with 1 mg/l 2-4D + 1.5 mg /l BAP.

Chemicals

All the chemicals used were of analytical grade and purchased from Hi Media from Hi-media Laboratory Pvt. Ltd. Mumbai.

Plant extract

Each of the dried and powdered test sample (10gm) (root and callus) was soaked in 200 mL methanol for several days at room temperature. The mixture was filtered and methanol was removed by rotary evaporator to give the crude methanolic extract. Procedure of extraction and evaporation was repeated three times. The dry methanolic extract was dissolved again in 20 mL methanol in separating funnel and mixed with 200 mL of 0.5 N sulphuric acid. A few drops of ammonia were added to the solution till the whole solution became basic (pH 9-12). 50 ml of chloroform was added in separating funnel and kept at room temperature for 24 hours. Discard the upper layer and taken the lower layer which was further evaporated to give the crude alkaloid mixture (Singh *et al.*, 2000), which was analysed for chromatographic and GC-MS analysis. The sample was analysed in Jawahar Lal Nehru University, Advance Instrument Centre, New Delhi.

GC-MS conditions

GCMS-QP 2010 Plus was used for identification and quantification of alkaloids, using MS libraries previously compiled from purchased standards. For the acquisition of an electron ionization mass spectrum, an ion source temperature of 250°C was used. The GC was equipped with a SE-30 capillary column a split injection piece (270°C) and direct GC-MS coupling (280°C). Helium (1.2 mL/min) was used as the carrier gas with a split ratio of 1:10. The oven temperature program for analyzing the extracts utilized an initial oven temperature of 100°C, maintained for 2 min, followed by a steady climb to 200°C at a rate of 7°C/min allowed to increase to 190°C at a rate of 30°C/min. This oven temperature was again maintained at 190°C for 5 min and then allowed to increase to 300°C at a rate of 7°C/min. This oven temperature was maintained for 2 min and finally ramped to 300°C at a rate of 10°C/min and maintained for a further 22 min. Injection temperature was 270° C and volume 250°C and 1 µL, respec-

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tively. The total GC running time was about 43.28 min. The MS operating conditions were as follows, Interference temperature of 260°C, Ion source temperature of 250°C, mass scan (m/z)-40-450, solvent cut time 7 min, scan speed 2000 amu/s total MS running time-50.28 min and Threshold -1000.

Identification

GC-MS is a valuable aid for identifying unknown peak as well as for confirming the identification of identified phytoconstituents. In some cases when no identical spectra were found, the structural type of the corresponding component was suggested only on the basis of its mass spectral fragmentation and retention data. Identification of components was based on directs comparison of the retention times and mass spectral data with those for standard compounds and computer matching with the library (Wiley library, NIST data bank, database NIST 98) as well as by comparison of the retention time those reported in the literature and mentioned in (Table-1,2).

Result and Discussion

In the present study a GC-MS procedure was applied for the identification of alkaloids in the root and callus of *W. somnifera*. The stereochemistry of these alkaloids could establish by MS data. It was suggested on the basis of their retention data reported by NIST library. Seventeen alkaloids from the *W. somnifera* have been detected by GC-MS. Alkaloids present are withasomnine:(4-phenyl-1,5-timethylene pyrazole); somniferine:[1-(3-4—dimethoxybenzyl)-6-7-dimethoxyiso-quinoline]; isopelletierine: 1(2-piperidinyl)2-propanone; anaferine: pyrazole[(1,3-bis,2R-piperidin-2-yl) propan-2-one)]; anahygrine: [1-amino-2(methoxymethyl) pyrrolidine]; tropine(3-end)-8-methyl-8 azabicyclo (3.2.1)octane-2-carboxylate; pseudotropine [Methyl (1R,2R,3S,5S)-3-(benzoyloxy)-8-methyl-8-azabicyclo (3.2.1) octane-2-carboxylate; Iron-pseudotropine: [Fe(1R,2R, 3S,5S)-3-(benzoyloxy)-8-methyl-8-azabicyclo(3.2.1)octane-2-carboxylate; withanine: 3 α -glyoxy-tropane; choline; cuscohygrine; N-(3-acetylphenyl)-1-Adanantanecarbox-amide; 1-[(5-Nitro-2-furfurylidene) amino]; oxacyclohexadecane-2,13-dione,13-oxime;scopoletin: Ashwagandhine:2,4-imidazolidiendione1-[{(5-nitro-2-furanyl)methylene]amino} (Table-1). Maximum number of alkaloids were present in roots (18) > callus (13). The GC-MS chromatograms of alkaloids of root and callus are shown in (Table-1; Fig.-1, 2).

Table 1: Retention time, molecular weight, molecular formula and major peaks of alkaloids detected by GC-MS of *W.somnifera*

S. No.	Compounds	Root	Callus	R.T. Min	Mol. Weight	Mol. Formula	Peak (m^+/Z)
1.	Choline(1-hexanol, 2 ethyl, 3 propane)	+	-	7.832	186	$C_{11}H_{22}NO_2$	15
2.	Cuscohygrineitosterol	+	-	7.957	212	$C_{10}H_{12}N_2$	19
3.	Withanine	+	+	8.433	178	$C_{12}H_{20}N_2$	22
4.	N-(3-Acetyl phenyl) 1-1-Adanantane Carboxamide	+	+	9.63	297	$C_{19}H_{23}NO_2$	27
5.	Anaferine-pyrazole [(1,3-bis,2R-piperidin-2-yl) propan-2-one]	+	+	9.925	224.3	$C_{13}H_{24}N_2O$	22
6.	Ashwagandhine	+	-	10.64	238	$C_8H_6N_4O_5$	42
7.	Isopellertierine 1(2-piperidinyl)-2-propanone	+	+	11.13	141.2	$C_{10}H_{12}N_2$	30
8.	3-a-glyoxytropane	+	+	12.13	353	$C_{19}H_{23}NO_4$	24
9.	Anahygrine [1-amino-2(methoxy methyl) pyrrolidine]	+	+	13.11	130.2	$C_6H_{14}N_2O$	21
10.	Scopoletin	+	+	13.28	192.17	$C_{10}H_8O_4$	15
11.	Iron Pseudotropine [Fe(1R, 2R, 3S, 5S)-3-(benzoyloxy) 8-methyl-8-azabicyclo (3,2,1)octane-2-carboxylats.	+	+	13.87	372	$C_{17}H_{20}NO_4Fe$	32
12.	Oxacyclohexane 213-Dione-13-oxime	+	-	13.91	270	$C_{15}H_{27}NO_3$	46
13.	Tropine(3-eno)-8-methyl-8-azabicyclo (3,2,1) octane-2-carboxylate	+	+	18.67	142	$C_{18}H_{15}NO$	14
14.	Pseudotropine[Methyl (1R, 2R, 3S, 5S)-3-(benzoylxy)-8-methyl-8-azabicyclo (3,3,1)octane-2-carboxylate	+	+	21.89	303	$C_{17}H_{21}NO_4$	20
15.	Withasomine (4-phenyl-1,5-trimethylenepyrazole)	+	+	22.45	184	$C_{12}H_{12}N_2$	44
16.	Somniferin[1-(3-4-dimethoxy benzyl)-6-7-dimethoxy-isoquinoline]	+	+	23.93	339.9	$C_{20}H_{22}NO$	24
17.	1-[(5-Nitro-2-furfurylidene) amino]	+	+	25.99	232	$C_8H_{12}NO$	13

Composition was estimated on the basic of calculation of the GC peak areas in percent by setting the total peak areas to 100 %. Maximum accumulation of withasomine was in roots (18.12%) while in callus it was found to be 10.72%. In callus cultures, presence of alkaloids indicates that capacity of synthesis of specific compounds is usually retained during culture (Hiraoka and Tabata, 1974). Maximum accumulation of isopellertierine was found more in callus cultures (6.58%) as compared to other root (4.37%). Whereas Root contains four alkaloids are 3 α glyoxytropane; 1-[{(5-nitro-2-furanyl) methylene]amino}; Ashwagandh-ine-2,4-imidazolidiendione-1-[{(5-nitro-2-furanyl) methylene]amino}; oxacyclohexadecane-2,13-dione, 13-oxime and their concentration identified by GC-MS was 8.26%, 4.56%, 2.05% and 1.59% , these alkaloids are not identified in callus. Most of the alkaloids which are identified in roots are also present in callus so for production of alkaloids for different uses can be purified through callus culture also, but the callus must be of 6-8 week old for better results (Table-2 and fig.-3,4). So, metabolomic fingerprinting of herbal extracts is desirable to standardize drugs and to establish the scientific basis of their pharmacological action.

Table 2 : Identified alkaloids from *W.somnifera* presented as % area by setting the total peak area to 100%

S. No.	Compounds	Root* (%)	Callus* (%)	R.T. Min	Mol. Weight	Mol. Formula	Peak (m^+/Z)
1.	Anaferine - Pyrazole[1,3-bis(C2R)-piperidin-2-yl] propan-2-one	10.12	8.14	9.925	224.3	$C_{13}H_{24}N_2O$	57

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2.	Ashwagandhine 2,4-Imidazolidine dione, 1-[{(5-Nitro-L-Furanyl) methylene}Amino]	2.05	-	10.64	238	$C_8H_6N_4O_5$	42
3.	Isopellertierine-1(2-piperidinyl) 2 propanone	4.37	6.58	11.13	141.2	$C_8H_{15}O$	30
4.	3- α -glyoxytropane	8.26	-	12.13	353	$C_{19}H_{23}NO_4$	24
5.	Anahygrine [1-Amino-2(Methoxy Methyl) pyrrolidine]	9.44	6.12	13.11	130.2	$C_6H_{14}N_2O$	21
6.	Iron Pseudotropine [Fe, methyl (1R1, 2R1, 3S, 5S)-3 (benzoyloxy)-8-Methyl-8-azabicyclo(3,2,1) octane-2-carboxylate	2.75	2.20	13.87	372	$C_{17}H_{20}NO_4Fe$	32
7.	Onacyclohexane 2,13-Dione-13-oxime	1.59	-	13.91	270	$C_{15}H_{27}NO_3$	40
8.	Tropine : (3-end)-8-methyl-8-azabicyclo (3-2-1) octane-2-carboxylate	10.47	8.21	18.67	142	$C_8H_{15}NO$	14
9.	Pseudotropine: Methyl (1R1, 2R1, 3s, 5s)-3-(benzoyl)-8-methyl-8-azobicyclo(3-2-1)octane-2-carboxylate	10.77	6.11	21.89	303	$C_{17}H_{21}NO$	20
10.	Withasomnine[4-phenyl-1,5-trimethylene Pyrazole	18.12	10.72	22.45	184	$C_{17}H_{21}NO$	44
11.	Somniferin-1-(3-4-dimethoxy benzyl)-6, 7-dimethoxyisoquinoline	8.14	7.92	23.93	339.9	$C_{17}H_{21}NO$	24
12.	1-[(5-Nitro-2-furfurylidene-amino]	4.56	-	25.99	232	$C_{17}H_{21}NO$	13

Conclusion

From the present study conducted, it can be concluded that the roots and callus of Withania somnifera differ in their chemical characteristics. The content of tropane alkaloids is higher in roots than callus. 17 alkaloids were present and withasomine was main alkaloid in roots (18.12%) and callus (10.72). Accumulation of isopellertierine was found more in callus culture (6.58%) as conditions may be favoured. Callus from nodal explants was proved to be a substantial source for scopolamine and other alkaloid production. So this species may find important application in medicinal treatment with its high alkaloid content.

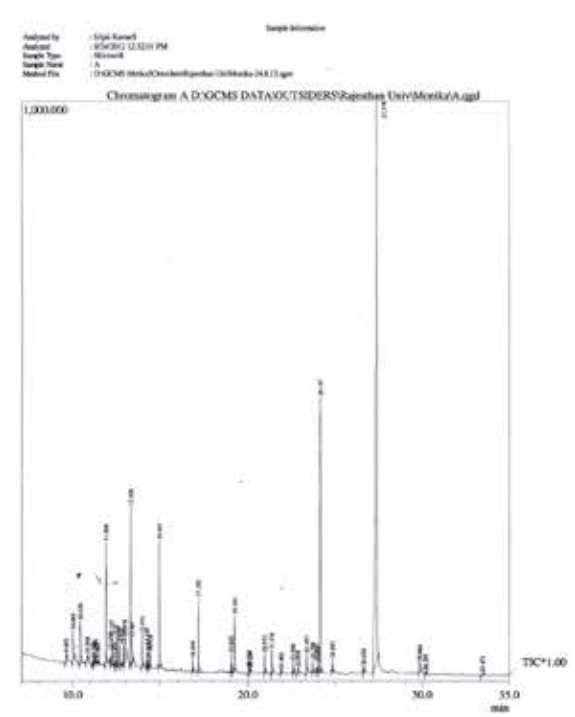

Figure 1: GC-MS chromatogram of methanolic extract present in *W. somnifera* (roots)

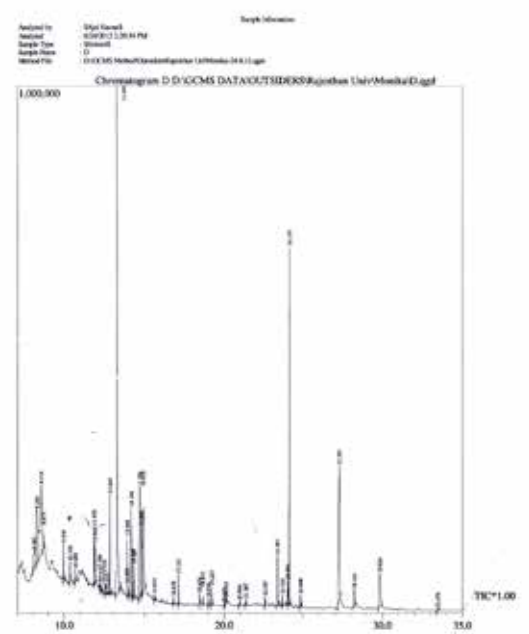

Figure 2: GC-MS chromatogram of methanolic extract present in *W. somnifera* (callus)

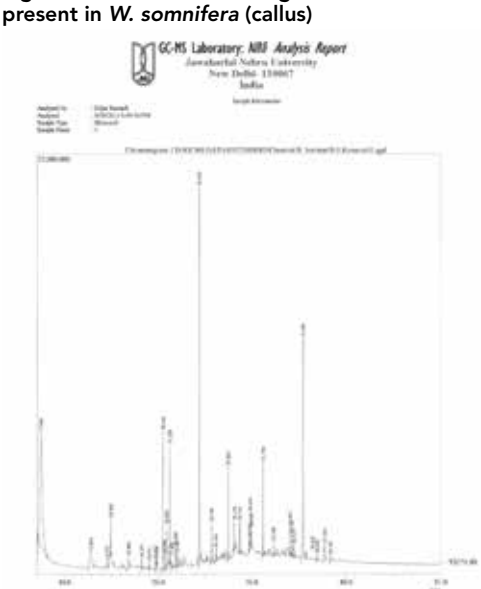

Figure 3: GC-MS chromatogram of alkaloids present in *W. somnifera* (roots)

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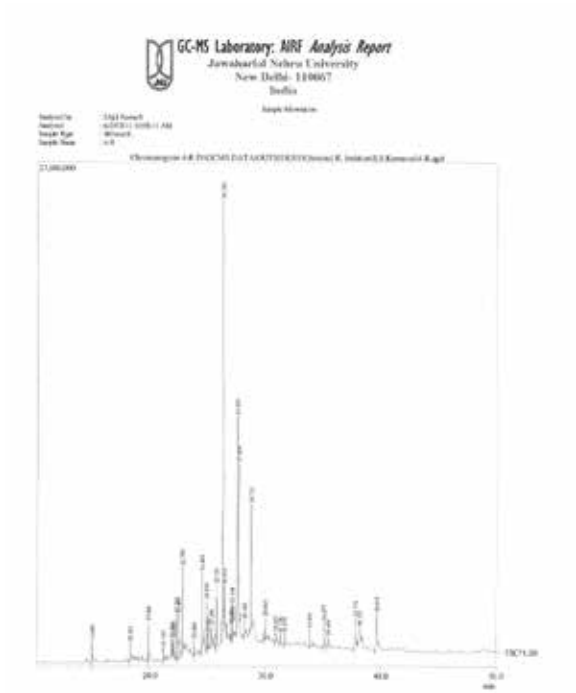

Figure 4 : GC-MS chromatogram of alkaloids present in *W. somnifera* (callus)

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