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RESEARCH ARTICLE

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A SIMPLE METHOD TO PURIFY WITHANOLIDE A FROM THE ROOTS OF *Withania somnifera* DUNAL

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ABSTRACT

Withania somnifera, commonly known as Ashwagandha, is a valued herb in Ayurvedic medicine. Roots, leaves and preparations of the plant are traditionally used as tonic, hypnotic, sedative and diuretic. *W.somnifera* mainly contains withanolides which are specific to the Solanaceae family. Withanolides are biologically active secondary metabolites present in roots and leaves of *W.somnifera*. In the present study, we have standardized the protocol for the extraction and purification of Withanolide A from the dried *in vivo* root of *W.somnifera*. Withanolide A is having a high medicinal value and possesses potent anti-tumor and antioxidant properties. This study involves a detailed investigation about the accumulation of secondary metabolite content in the *in vivo* root both quantitatively and qualitatively extracts the pure compound using the simple techniques viz., Thin Layer Chromatography, Column Chromatography and High Pressure Liquid Chromatography.

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KEY WORDS

Withania somnifera roots, Simple Techniques, Pure compound, Withanolide A, High Pressure Liquid Chromatography.

INTRODUCTION

Medicinal plants are a tremendous source of raw material for the modern drug industry. Since time immemorial, plants have been extensively exploited for their therapeutic property. Whole plant or parts of plants were the main components of folk or ethanomedicine, practiced in India and other parts of the world like China, Middle East Africa and South America[1]. *Withania somnifera*, also known as Ashwagandha, Indian ginseng is an important medicinal plant, which is cultivated in India for the medicinal purposes. The roots of the plant are categorized as rasayanas, which are reputed to promote health and longevity by augmenting defense against disease, arresting the ageing process, revitalizing the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and by creating a sense of mental well being[2].

The roots and leaves of Ashwagandha contain various alkaloids, viz., withanolides[3], withaferins[4] and withanosides[5]. The withanolides are steroidal compounds and bear resemblance, both in action and appearance to the active ginsenosides of Asian ginseng. Studies show that the plant has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, antitumor, astringent and more recently to treat ulcers, bacterial infection, venom toxins and senile dementia. The biological activities of withanolides, especially of the dominant withanolide A and withaferin A, have been studied extensively and, more recently, have been shown to have anti-cancerous activity[6,7]. In this paper, we discussed about the purification of the secondary metabolite from the *in vivo* root extract of *W.somnifera* by simple techniques for the effective elution of a single compound withanolide A.

MATERIALS AND METHODS

(i) Plant material

Roots of *W.somnifera* were brought from the medicinal plant market in Maharashtra in 2008. The roots were air dried at room temperature and powdered mechanically. The powdered roots were used as the plant material for all analysis.

(ii) Extraction procedure

The extraction of secondary metabolites was carried out following a modified method of Bandhoria et al[8]. The dried and powdered materials (20 g) were extracted with 800ml 50% ethanol by sonication for 20 mins at room temperature. The ethanolic extracts were evaporated in a water bath at 40°C. The aqueous layer from the ethanolic extracts was subjected to sequential extraction with chloroform, ethyl acetate and n-butanol. The extracted fractions were evaporated to dryness in a flash evaporator (Roteva - Equitron, Make). The residues obtained were redissolved in HPLC grade methanol. Chloroform fraction was used for silica gel column chromatography. Silica gel (100-200 mesh, size) was used for packing the column with chloroform as packing solvent and eluted with chloroform and methanol at different ratios.

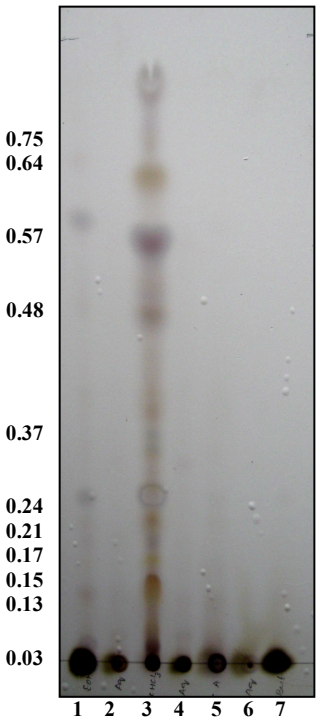
The obtained single compound were performed with a gradient High Pressure Liquid

Chromatography (Shimadzu HPLC, Model Class VP series version 6.1 software) to confirm the single compound as withanolide A. The system was equipped with photo diode array detector SPD M10A *VP*, CTO- 10AS *VP* column oven, CBM-10A *VP* system controller was carried out by following the method of Ganzera et al[9]. Separation was carried out with reverse phase C18 phenomenex column (250 mm × 4.6 mm) with reagent 1% Trifluroacetic acid containing Water and methanol (Rankem, India) as an eluant at a flow rate of 1ml min^{-1} and the column temperature was maintained at room temperature. Withanolide A standard was obtained from Chromadex (Laguna Hills, CA, USA). Validation of quantitative method was performed with samples for four injections of 20µl each.

RESULTS AND DISCUSSION

The TLC profile for the sequential extraction of the *in vivo* roots of *W.somnifera* as shown in figure 1, chloroform fraction showed more spots than the other two organic fractions since; chloroform fraction was subjected to further analysis. The physical appearance of the extracts described in table 1.

Figure 1
TLC profile for Sequential extraction of *Withania somnifera* Dunal., root



Note: Lane 1 - 50% Ethanol extract, Lane 2 - Aqueous extract, Lane 3 - Chloroform extract, Lane 4 - Aqueous extract, Lane 5 - Ethyl acetate extract, Lane 6 – Aqueous extract, Lane 7- n-Butanol extract

Table 1
Physical appearance of the sequential extraction

S.No	Sequential extraction	Colour of the extract	Consistency
1	Ethanol (50%)	Yellowish brown	Clear liquid
2	Aqueous (after evaporation of ethanol)	Dark brownish yellow	Turbid
3	Chloroform (100%)	Yellowish brown	Clear liquid
4	Ethyl acetate (100%)	Pale yellow	Clear liquid
5	n- Butanol (100%)	Pale yellow	Clear liquid
6	Aqueous(after evaporation of organic solvents)	Light yellowish brown	Clear liquid

Jamal et al[10] obtained pure compounds by employing chloroform as the solvent for extraction of withanolides. Therefore, chloroform fraction from the sequential extraction of *in vivo* stored roots was used for the elution of withanolides by the column chromatographic method in the present study, assuming that it will result in the successful purification of single withanolide. Also Malik et al[11] isolated markers in a purified form, from the chloroform extracts of the roots. These markers were withanolides/glucowithanolides were used in chemoprofiling of the bioactive extract.

The purification of a single compound from the chloroform fraction was obtained by the eluants as chloroform and methanol in different ratio (shown in table 2). The column eluted fractions were air dried and run in TLC containing the mobile phase as toluene: ethyl acetate: formic acid in the ratio (5:5:0.1) and 10% sulphuric acid as the spraying reagent. The obtained single compounds were pooled and crystallized.

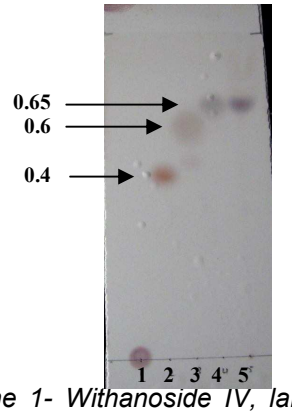
Table 2
The eluant ratio for Silica gel Column Chromatography

	Chloroform : Methanol (ml)		
	Column 1	**Column 2**	**Column 3**
Silica gel Column (the eluant ratio of solvents for each column)	100:0, 90:10, 80:20… 0:100	100:0, 99:1, 98:2… 90:10	100:0, 99.8:0.2, 99.6:0.4… 99.0:1.0
Ratio of the eluants obtain a single compound	90:10	99:1 and 98:2 obtained samples were pooled and packed for column 3, since they contained group of compounds were eluted together	99.8:0.2

Silica coated TLC is convenient and suitable for the analysis of withanolides. It is often used to monitor fractions or finally purified withanolides. Chloroform - methanol (95:5) is frequently used solvent system for aglycones[12] and chloroform - methanol (90:10) for glycosides[13]. The obtained purified compound is expected to be withanolide glycoside. So the mobile phase for TLC of sample and standards were changed as chloroform: methanol in the ratio 9:1 with same spraying reagent. The single compounds were analyzed in TLC with standards Withaferin A, Withanolide A, Withanone and Withanoside IV. The TLC screening of purified compound and standard withanolide A showed the same R_f value as 0.65. From this result it can be stated that the purified compound is withanolide A (shown in figure 2). The purified compound in chloroform fraction was shown in figure 1 (lane 3).

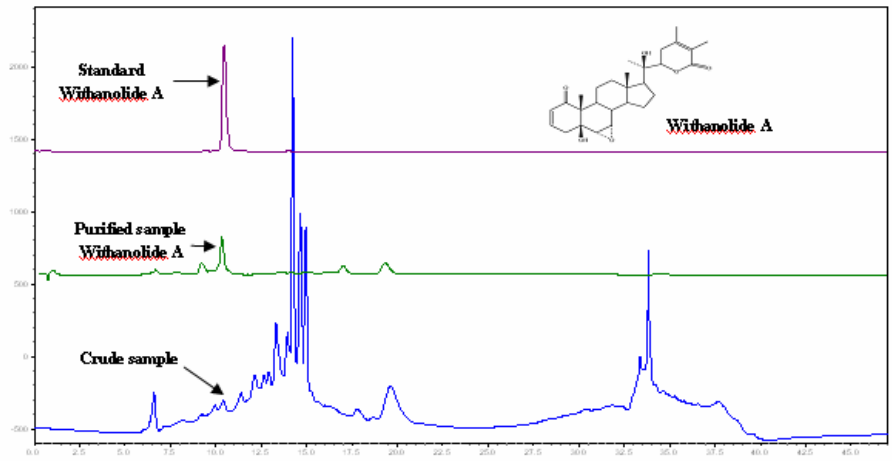
Figure 2

The TLC profile of Pure Compound with standards



Note: Figure 2 - From the TLC profile lane 1- Withanoside IV, lane 2 - Withanone, lane 3 - Withaferin A, lane 4 - Withanolide A and lane 5 - Purified compound.

The purified compound was further confirmed by High Pressure Liquid Chromatography analysis with standard Withanolide A as reference compound. Both purified compound and standard obtain peak at same retention time (10.4th mins) as shown in figure 3.



Note: The arrow in figure 3 shows the Withanolide A compound peak in chloroform fraction, purified compound and standard

CONCLUSION

From the present study, it can be concluded that the roots of *Withania somnifera* that contain Withanolide A compound were eluted using simple techniques with less cost effect and they are quantified with the HPLC techniques. So, the obtained Withanolide A compound will be used as the marker for analyzing the unknown compounds. 1g of the root powder obtains approximately 50µg of Withanolide A compound from the dried roots of *Withania somnifera.*

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