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Evaluation of the phytochemical constituents in ADJ6, an anti-diabetic polyherbal formulation by GC-MS

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Abstract
Background: According to WHO guidelines, standardization and evaluation of herbal drug are very essential to elucidate various properties such as key phytoactive components. Thus it is very vital for the herbal drug preparations to undergo stringent evaluation procedures in order to develop it in to a viable product for the global market.
Objective: Our present study focuses on screening of the polyherbal formulation ADJ6 for the presence phytochemical constituents using GC-MS.
Materials and Methods: Initially, Phytochemistry of ADJ6 ethanol and diethyl ether extract was determined using TLC plates and sprayer reagents. GC-MS analysis of ADJ6 was performed on GC system coupled with mass spectrometry.
Results: The phytochemical analysis showed presence of phenols, aminoacids/proteins, anthrones, tannins and terpenes in ethanolic extract while diethyl extract showed presence of terpenes and sugars. GC-MS Analysis of both the extracts revealed the presence of key phytochemical constituents. A total of 17 compounds were identified 10 and 7 compounds in ethanol & diethyl ether extract respectively.
Conclusion: From our present study, it is evident that ADJ6 contains various phytochemical components which possess Antihyperglycemic property. However further studies should be conducted to elucidate the anti-hyperglycemic property of ADJ6.

Keywords: ADJ6, GC-MS, TLC sprayer method.

1. Introduction
Ancient Indian System of Medicine especially *Ayurveda* has been one of the oldest systems of medicine (articulated around 1500BCE, *Atharva Veda*) known to mankind [1]. The ancient system of medicine highlighted the importance of maintaining optimistic lifestyle through appropriate practices [2]. The main importance for maintaining good lifestyle includes taking the right form of food which is conceptualized to have composed of five elements namely Prithvi (Earth), Jala (Water), Teja (Fire), Vayu (Air) and Akash (Space). Hence food is one of the basic elements required for better living hence Ayurveda emphasizes on dietary guidelines since it has more influence on physical and mental development of an individual [1]. The ancient scholars put an emphasis on preparing Ayurvedic and herbal preparations with utmost care through specific pharmaceutical techniques such as *shodhana*, *jarana*, *marana* etc. that include detoxifying methodologies [3]. In this modern age, a Greek Pharmacist and Physician named Galen had reported that herbs not only possess beneficiary effects but may comprise components that can be harmful [4]. Hence it is essential to standardize herbal derived products using various testing methodologies including assessment of various bioactive components, physical and chemical parameters and system based toxicity assays [5].
Use of plants and plant products has been increased in recent times due to its natural source & ensures safe in biological activity. Nevertheless, some plants or plant products may not be reactive, some interact with other drugs and some cause toxic health effects. Hence it is necessary to adhere to the guidelines & safety measures for standardization of the herbal drug evaluation parameters [6]. In recent times, Gas chromatography - Mass Spectrometry has become a key technique for assessing the phyto-active components in plants and various other components [7]. The formulation ADJ6 has already been studied for its *in vitro* inhibitory activity against key digestive enzymes α-amylase and glucosidase [8]. The present study focuses on assessing the phytochemical constituents through Gas α-chromatography – Mass Spectrometry technique.

2. Materials and Methods

Chemicals

Ethanol and diethyl ether were of HPLC grade and were purchased from Fisher Scientific, Mumbai, India. All other laboratory chemicals were of analytical grade and were purchased locally in Chennai, India.

Collection of plant

In total, 6 different plant parts have been used for the development of formulation and have been discussed in detail [8]. The plants used for the formulation were (*Momordica charantia, Psidium guajava, Phyllanthus emblica, Trigonella foenum-graecum, Syzygium cumini* and *Gymnema sylvestre*). All the plants were collected from the medicinal farm of Frontier Mediville (Elavur, Gummidipoondi, India) and were submitted to the Plant Anatomy Research Centre (Tamil Nadu, India) for authentication. The voucher specimen numbers have been provided as an additional file. The individual plants were minced using a mixer and immediately freeze dried to prevent the loss of bioactive components. All the six components were mixed proportionately in a specific combination and stored in air tight containers to prevent moisture until use.

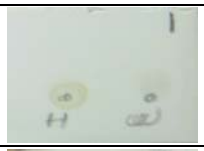
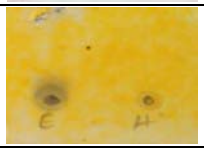
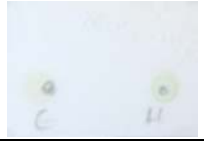
Preparation of ADJ6 extract

10 g of ADJ6 powder was immersed in 100 ml ethanol and 100 ml diethyl ether, separately and left overnight in an orbital shaker. Then the solvents were filtered through Whatman No.1 filter paper (0.22 µ) and the crude ethanol and diethyl ether crude extract were obtained. The extract was concentrated in vacuum to dryness. The extract was used for GC-MS analysis.

Identification of Phytochemical Constituents using Sprayer Reagents

The various phytochemical constituents of the crude ethanol and diethyl extract of ADJ6 formulation was analysed using Thin Layer Chromatography (TLC) plate with some modifications [9]. The pre-coated TLC plates of size 5 cm × 2 cm^2 was used for the assay. The extracts were drawn with capillary tubes and applied as spots on a stationary phase (silica gel coated plates) about 1 cm from base. After incubating for 10 min the plates were sprayed with suitable chromogenic reagents and were observed using visible light, UV (254 & 364nm).

Table 1: Identification of Phytochemical Constituents using TLC - Sprayer reagents

S. No	Sprayer Reagent	Observation	Ethanol extract	Di-ethyl extract
1	1% Aluminium Chloride in Ethanol		ns[#]	ns
2	Anisaldehyde – sulphuric acid		Appearance of Violet colour; presence of phenols	Appearance of grey, green colour; presence of terpenes and sugars
3	Ninhydrin reagent		Appearance of reddish spot; presence of aminoacids / proteins / amines	ns
4	Dragondroff's reagent		ns	ns
5	10% Potassium hydroxide		Appearance of Yellow colour; presence of hydroquinone derivative anthrones	ns
6	10% Ferric Chloride reagent		Appearance of Blue or green colour; presence of tannins	ns
7	Pancal D reagent		(+) Presence of terpenes	ns
8	Concentrated Sulphuric acid		ns	ns

Footnotes: [#] ns - no significant colour change; E- ethanol extract; H – diethyl ether extract

Table 2: Compounds identified in the ethanolic & diethyl extract of ADJ6

S. No	Name of the Compound	Retention Time (RT)	Molecular Weight	Molecular Formula	% Peak Area	Nature of Compound
ADJ6 ethanol extract						
1	5-(p-Aminophenyl)-4-(p-tolyl)-2-thiazolamine	4.45	281.38	$C_{16}H_{15}N_3S$	6.26	Thiazole
2	5-Hydroxy-1-(3-isopropoxy-propyl)-2-methyl-1H-benzo(g)indole-3-carboxylic acid methyl ester	6.05	355.434	$C_{21}H_{25}NO_4$	1.77	Indole
3	2,5,8-Triphenyl benzotristriazole	7.73	429.447	$C_{24}H_{15}N_9$	0.33	Benzotriazole
4	Cholestan-3-one,2-(1-hydroxy-2-(3-methylphenyl)ethyl)-	9.38	502.6121	$C_{36}H_{56}O_2$	27.92	Cholestane
5	Cholestane,2-formyl-3-(2-methylbenzylidene)-	13.1	502.6121	$C_{37}H_{56}O$	5.12	Cholestane
6	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	14.12	354.7944	$C_{27}H_{42}O_4$	3.53	Androstane
		15.07	354.7944		3.61	
7	(3,4-Dimethyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-(4,4-dimethyl-5-(2,3,3-trimethyl-5-methylthio-3,4-dihydro-2H-pyrrol-2-ylmethylene)pyrrolidin-2-ylene)-thioacetic acid, S-(tert. butyl) ester	9.32	502.6121	$C_{27}H_{41}N_3O_2S_2$	27.44	Pyrrole
8	2-Acetylamino-5-iodo-4-p-tolyl-thiophene-3-carboxylic acid ethyl ester	7.5	428.6687	$C_{18}H_{19}IO_3$	9.40	Thiophene
9	4-(4-Ethoxycarbonylbuta-1,3-dienyl)-1-methyl-2,5-diphenyl-1H-pyrrole-3-carboxylic acid, ethyl ester	11.98	428.6832	$C_{27}H_{27}NO_4$	5.49	Pyrrole
10	18,19-Seco-15a-yohimban-19-oic acid, 20,21-didehyro-16a-(hydroxymethyl)-,methyl ester	10.75	354.7679	$C_{20}H_{24}N_2O_3$	9.13	Yohimbine
ADJ6 Diethyl Extract						
11	Acetic acid,2-(1,5-dimethyl-3-phenylthio-2-indoyl)-ethylester	10.85	339.45	$C_{20}H_{21}NO_2S$	3.07	Indole
12	Acetic acid,4,4,6a,6b,8a,11,11,14b-octamethyl-13-oxodocosahydropicen-3-yl ester	13.15	484.765	$C_{32}H_{52}O_3$	5.10	Picene
13	Yohimbic acid	14.17	340.423	$C_{20}H_{24}N_2O_3$	46.21	Yohimbine
14	2-benzimidazolinone	9.75	194.19	$C_9H_{10}N_2O_3$	30.74	Benzimidazole
15	Cholest-2-eno(2,3-c)naphthalene,6'methyl-	9.43	484.812	$C_{36}H_{52}$	8.70	Cholestane
16	3-Butenenitrile,4-anilino-3,4-bis(4-quinolyl)-	16.27	412.496	$C_{28}H_{20}N_4$	3.50	Quinoline
17	2-(1-Styryl-7H-indeno91,2-f)quinolin-3-yl)phenol	12.08	411.504	$C_{30}H_{21}NO$	2.68	Quinoline

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

GC-MS analysis was performed at the SAIF, IIT-Madras, and Chennai, India. Ethanol and diethyl ether extracts of ADJ6, were subjected to GC and MS JOEL GC mate equipped with secondary electron multiplier (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m X 0.25 mm I.D. The experimental conditions were 20 min. at 100 °C, column temperature: 235°C for 3 min; injector temperature: 240 °C; carrier gas: helium; and split ratio: 5:4. 1µl of the sample was evaporated in a split less injector at 300 °C and the run time was 22 min. The phytochemically active components were identified by gas chromatography coupled with mass spectrometry. The spectrum of GC-MS was analysed using the NIST08 library which has more than 62,000 patterns.

3. Results and Discussion

The TLC analysis of the phytochemical constituents in the ADJ6 ethanol extract showed presence of phenols, amino acids, proteins, amines, anthrones, tannins and terpenes while the diethyl ether extract showed presence of terpenes and sugars (as shown in Table 1).

GC-MS Analysis of the ADJ6 ethanol extract showed presence of key phytochemically active constituents while the ADJ6 diethyl extract showed presence for vital compounds, respectively. The retention time (RT), molecular weight, % peak area and the nature of the compound are shown in Table 3. GC-MS analysis of ethanol extract showed 27.44% presence of (3,4-Dimethyl-5-oxo-2,5-dihydro-1H-pyrrol-2-yl)-(4,4-dimethyl-5-(2,3,3-trimethyl-5-methylthio-3,4-dihydro-2H-pyrrol-2-ylmethylene)pyrrolidin-2-ylene)-thioacetic acid, S-(tert. butyl) ester and 27.92% presence of Cholestan-3-one,2-(1-hydroxy-2-(3-methylphenyl)ethyl)- which are of pyrrole and cholestane derivatives. Other compounds that were present are 5-(p-Aminophenyl)-4-(p-tolyl)-2-thiazolamine, 5-Hydroxy-1-(3-isopropoxy-propyl)-2-methyl-1H-benzo(g)indole-3-carboxylic acid methyl ester, 2,5,8-Triphenyl benzotristriazole, Cholestane,2-formyl-3-(2-methylbenzylidene)-, Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-, 2-Acetylamino-5-iodo-4-p-tolyl-thiophene-3-carboxylic acid ethyl ester, 4-(4-Ethoxycarbonylbuta-1,3-dienyl)-1-methyl-2,5-diphenyl-1H-pyrrole-3-carboxylic acid, ethyl ester and 18,19-Seco-15a-yohimban-19-oic acid, 20,21-didehyro-16a-(hydroxymethyl)-,methyl ester. While the diethyl ether extract of the ADJ6 showed 46.21% and 30.74% presence of Yohimbic acid and 2-benzimidazolinone respectively which are derivatives of Yohimbine and benzimidazolinone. The other compounds were Acetic acid,2-(1,5-dimethyl-3-phenylthio-2-indoyl)-ethylester, Acetic acid,4,4,6a,6b,8a,11,11,14b-octamethyl-13-oxodocosahydropicen-3-yl ester, Cholest-2-eno(2,3-c)naphthalene,6'methyl-, 3-Butenenitrile,4-anilino-3,4-bis(4-quinolyl)-, 2-(1-Styryl-7H-indeno91,2-f)quinolin-3-yl)phenol. The major derivatives were found to be Yohimbine, benzimidazole, Cholestane and Pyrrole. Other derivatives were present in a considerable amount.

Yohimbine has been reported for its potent alpha-2 adrenoceptor antagonist and as K_{ATP} inhibitor in pancreatic beta cells & may possess insulinotropic activity and hypoglycaemic effects [10]. Benzimidazolinone may be used for reducing and treating epilepsy [11] and also for relaxing of mesenteric arteries in diabetes [12]. Pyrrole derivatives are also being studied for their DPP-IV inhibitory effects and are being developed for Type 2 diabetes and obesity therapy [13]. Cholestane derivatives are being tested for their anti-cancer

effects [14-16]. The derivative compounds having more peak percentage during detection are alone discussed in detail. However other derivatives mentioned as in Table 5 & Table 6 have their beneficial effects in treating various disorders.

Our present study revealed the presence of therapeutically active compounds in ADJ6 formulation. Further toxicity and efficacy of ADJ6 will be evaluated using *in vivo* experimental models and it will be pursued to encourage the formulation to be used as a viable dietary supplement for diabetics.

In addition, future studies will involve isolation of the active ingredients to elucidate their pharmacological activity and will also help in further development of the herbal medicine and drug development.

The identification of phytochemical constituents of the polyherbal formulation using GC-MS has lead to the elucidation of various biological activities of compounds ranging from anti-tumour, anti-diabetic, insulinotropic, etc. Furthermore studies will be carried out to explicate the anti-hyperglycaemic property of the formulation using experimental animal models.

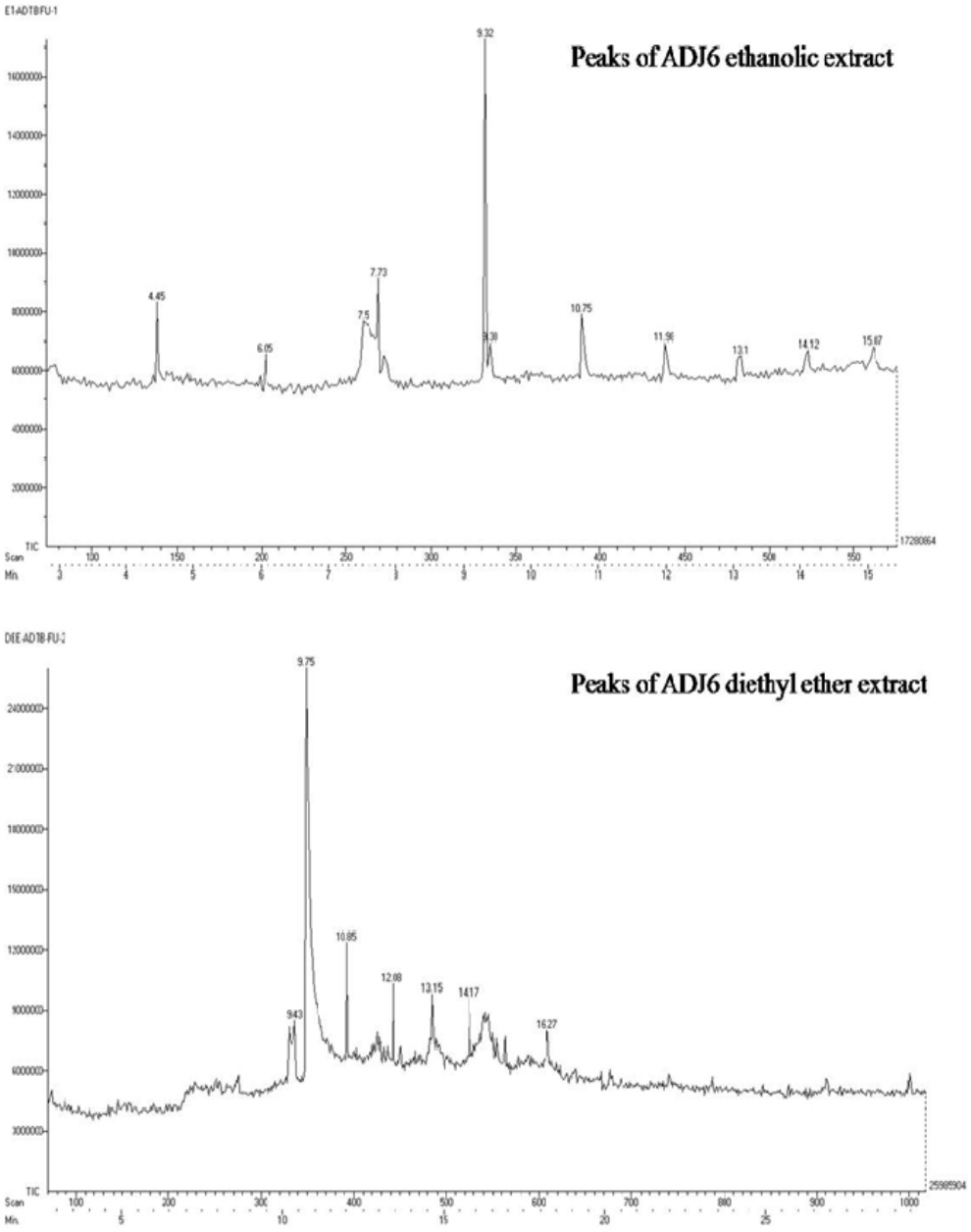


Fig 1: GC-MS chromatogram of ADJ6 ethanol and diethyl ether extract

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Conflict of Interest

The authors declare no conflict of interest.

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