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SEARCH FOR METFORMIN HYDROCHLORIDE (ANTIHYPERGLYCEMIC AGENT) IN WITHANIA COAGULENCE (FAMILY-SOLANACEAE) WITH THE HELP OF AN IMPROVED HPLC METHOD

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ABSTRACT

Metformin hydrochloride originally sold as Glucophage is an oral anti-diabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of type-II diabetes.Rapid drug development is required for the curing diabetes. An innovative analytical method of High Performance Liquid Chromatography(HPLC) has been used to establish the presence of Metformin hydrochloride in the test sample (Withania coagulens-Family:Solanaceae) . The findings reveal that Withania coagulens(Seeds) show anti hyperglycemic activity due to the probable presence of Metformin hydrochloride which might be the active principle. The result was quite encouraging as (57.79% of Metformin hydrochloride). The test sample may be analyzed in detail further. There is no doubt, if the process of drug development is achieved for Withania coagulens-Seed, a new therapy for diabetes from non- conventional sources will be established. So far other time taking processes like Thin Layer Chromatography etc were being used for this purpose. This process is fast, more accurate and a very minute quantity of the sample is required. With a very limited sample quantity and sample concentration, HPLC offers the best solution for the analysis. The method will be useful in creating extensive database of active components of the various plant species. By using these chemical database better drug design can be achieved for the treatment of diabetes. Chemi-informatic exercises have been demonstrated as a proof of concept for the same.

Biography: Dr Shipra Roy is Ph.D. from University of Allahabad, Allahabad, India in 1986 at the age of 27 years. She is faculty at Government Geetanjali Girls Postgraduate Autonomous College, Bhopal, India. She has one United States Patent and four Indian Patents in her name. Two new Patent applications have been filed in Indian Patent Office. Her field of research is Green Chemistry. She is in continuous research since 1981.
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I. INTRODUCTION

Diabetes is probably the fastest growing metabolic disease in the world and as knowledge of the heterogeneous nature of disease increase so does the need for the more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of the diabetes (Akhtar and Ali, 1984) but only a few

have been scientifically evaluated. Historical literatures reveal that knowledge regarding diabetes existed since Brahmic period as this was mentioned in Ayurvedic text books-Sushruta samhita written in Fourth and Fifth Centuries B.C (Dhanukar and Thatte, 1989). In this ancient text, two forms of diabetes were described: one genetically based and the other as a result of dietary indiscretion (Dhanukar and Thatte, 1989). Even the treatment in the Indian ancient pharmacopoeia mentioned specific treatments for the two types, including **dietary modifications, medicinal plant remedies and minerals.** Moreover, the research conducted over last several decades has shown that plant and plant based therapies have potential to control and treat diabetes (Oliver and Zahnd, 1979; Bailey and Day, 1989; Ivorra *et al.*, 1989; Marles and Farnsworth, 1995) and its complications (Grover *et al*., 2001). Role of Indian medicinal plants having antidiabetic activity has also been reviewed by (Grover *et al*. 2002).

Screening these plants for hypoglycemic (blood sugar lowering) activity has shown that 81% yielded positive results. This figure reflects the "great variety of possible active constituents and mechanisms of action," and illustrates the excellent potential for discovering new sources of anti diabetic drugs. Promising leads reflecting this wide range of anti diabetic constituents and mechanisms of hypoglycemic activity are being studied. Continuing to fight hyperglycemia, the efficacy of these therapeutic agents is compromised in several ways. Individual agents act only on part of the pathogenic process and only to a partial extent. This may be the reason that even after so much advancement in understanding the disease process and availability of a wide range of therapeutic agents, the disease is still progressing. Noor *et al*. (2008) reported the antidiabetic activity of *Aloe vera* in streptozotocin (STZ) induced diabetic rats. Noor *et al*. (2008) have also mentioned that there are two possible explanations for this finding. First, *A*. vera may exert its effect by preventing the death of $ cells and/or second, it may permit recovery of partially destroyed $ cells. Burcelain *et al*. (1995) reported that the hypoglycemic action of the extract of herbal plants in diabetic rats may be possible through the insulinomimictic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles.

The ethnopharmacological use of herbal remedies for the treatment of diabetes mellitus is an area of study ripe with potential as a starting point in the development of alternative, inexpensive therapies for treating the disease. There are over thirty million people today suffering from one form of diabetes or another and the numbers are increasing throughout the world. Researchers have published an extensive review of plants with reported antidiabetic activity in order to provide the preliminary information needed to design research whose goal is to develop "indigenous, renewable, medicinal plant resources as practical cost-efficient alternatives." Therefore, an exhaustive survey of literature has been carried out.

The subject of phytochemistry, or plant chemistry, has developed in recent years as distinct discipline, some where in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function. One of the challenges of phytochemistry is to carry out all the above operations on vanishingly small amount of the material. Frequently, the solution of a biological problem in, say, plant growth regulation, in the biochemistry of plant- animal interactions, or in understanding the origin of fossil

plants depends on identifying a range of complex chemical structures which may only be available for study in microgram amounts.

Plants are the only source of well- established traditional and modern drugs and phytochemicals surveys and documentation have been taken worldwide with a view to prepare the inventories for food, fiber medicine etc. During last few decades several research papers have been published on various aspects of ethno medicine. It is thought to be quite fruitful to study the world wide ethno botanical information about medicinal plants having hypoglycemic activity. India, treasure of plants, has wide variety of medicinal plant species, the therapeutic values of which have made history as the textual part of Indian literature. Rigveda and Atherveda are the oldest testimonials of Indian culture reflect the rational aptitude towards plants and their exploration for human welfare. After extensive survey of literature Withania coagulenswas selected for investigation. The basis of selection was easy availability and novelty of the findings..

1.1 Withania Coagulens

Image 2.1

Image 2.2

1.2 Family : Solanaceae

1.3 Part investigated: Seed

1.4 Worldwide use: India (Punjab & Gujrat) , Its a wild medicinal plant. This species occurs in the Hindu-Kush Himalayan range across, Afghanistan, Pakistan and India. Within India, it occurs in the drier parts of Punjab and Rajasthan, it has also been recorded from the region around Simla, Garhwal and Kumaun, Himanchal Pradesh, India.

1.5 Phytochemicals:

22 R,20β-hydroxy-1-oxowitha-2,5,24-trienolide (90),coajulin J (91), coajulin R-(92), coajulin U(93),(22R)-14,20-epoxy-17β-hydroxy-1-oxowitha-3,5,24-dieno-3β-(O-B-D-glucopyranoside)(94), Ajujin A (102), Methyl 1-4-benzoate (103), β-sitosterol (105), β-sitosterol glucoside (106), and β-amyrin(104).(Aniqa Naz et al, 2002).

II. MATERIAL & METHODS

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Based on the various observations a number of experiments were performed to develop the most accurate High Pressure Liquid Chromatographic method for the desired analytical study.

Efforts have been made to develop an accurate analytical method of HPLC for the desired study of presence of Metformin hydrochloride [chemically known as $C_4H_{11}N_5$.HCl 165.22 (Imidodicarbonimidic diamide, N,N-Dimethyl1-, monohydrochloride.1.1-dimethyl biguanide monohydrochloride)]

A sensitive and rapid high-performance liquid chromatographic assay is developed and validated for the determination was performed at ambient temperature by pumping a mobile phase of 0.03m Di-ammonium hydrogen phosphate buffer (pH 7, 250 ml) in methanol (750 ml) at a flow rate of 1 ml/min through a silica column. Metformin was detected at 236 nm, and eluted. No endogenous substances were found to interfere. (Chen-Hsi Chou et al, 2003)

III. METFORMIN HYDROCHLORIDE REAGENTS

Standard Metformin Hydrochloride 500 mg of Aristo pharma used. Methanol (HPLC grade) was used from merck, HPLC grade purified water from Rankem was used, and Diammonium hydrogen phosphate was used from Merck.

3.1 Instrument used-Thermo finnigan USA chromatographic system was used in this study, equipped with pump, UV detector, Auto injector , Column oven System controller, & class VP software.

3.2 Chromatographic Column

An isocratic mobile phase of 0.03M diammonium hydrogen phosphate buffer and Methanol (250:750), pH 7.0 was pumped at the flow rate of 1mL per min. Column was used as 3.9 X 150 mm, C-18 (ODS). The flow rate of the mobile phase was maintained at 1.0 ml/min. The detection wavelength was kept at 236 nm.

3.3 Standard preparation:500 mg Metformin hydrochloride was dissolved in HPLC grade purified water (Qualigens B.No 6912 6410T) and was diluted to 100 ml. in volumetric flask, to obtained 5000 ppm standard solution. This stock solution was further diluted ten times (10 ml in 100 ml volumetric flask) to obtained 500 ppm solution. Again 5 ml. of this solution was diluted to 100 ml. with HPLC grade water to obtained 25 ppm standard solution. This Solution was filtered through 0.2 µm Poly tetra fluoro ethylene (PTFE whattman) filter paper to obtained 25 ppm standard solution.

3.4 Sample preparation: 250 mg sample extract weighed and diluted to 50 ml. with HPLC grade purified water (Qualigens B.No 6912 6410T) Solution was filtered through 0.2 µm PTFE (whattman) filter paper.

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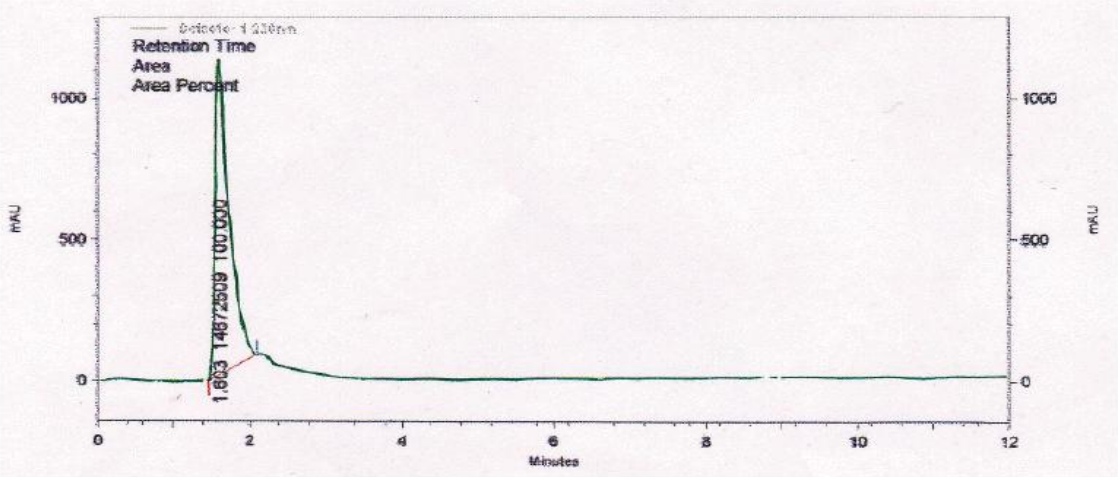
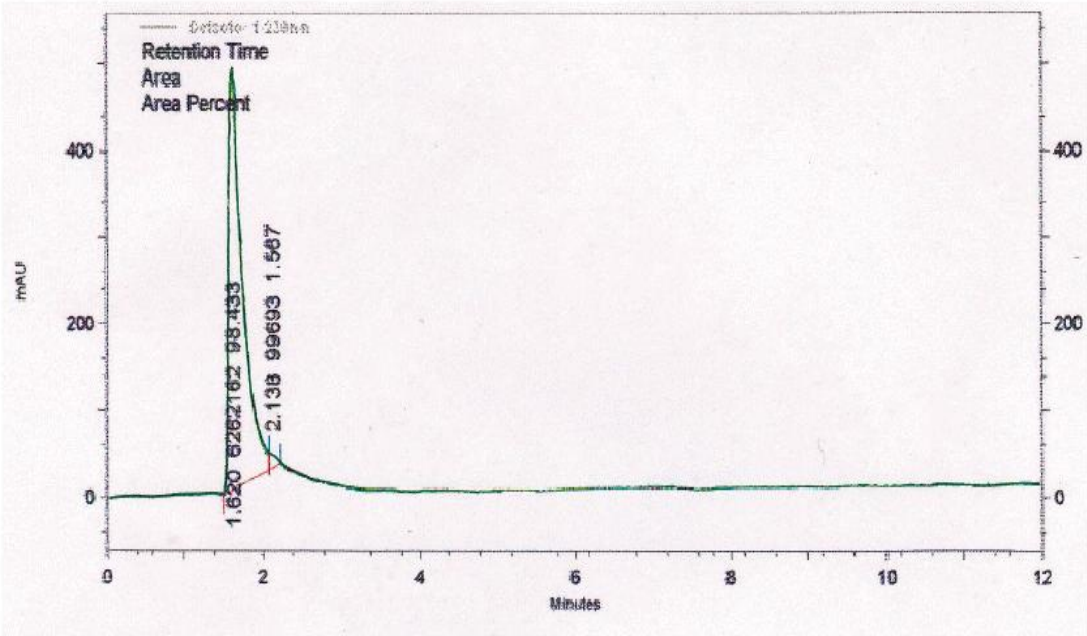
IV. OBSERVATIONS

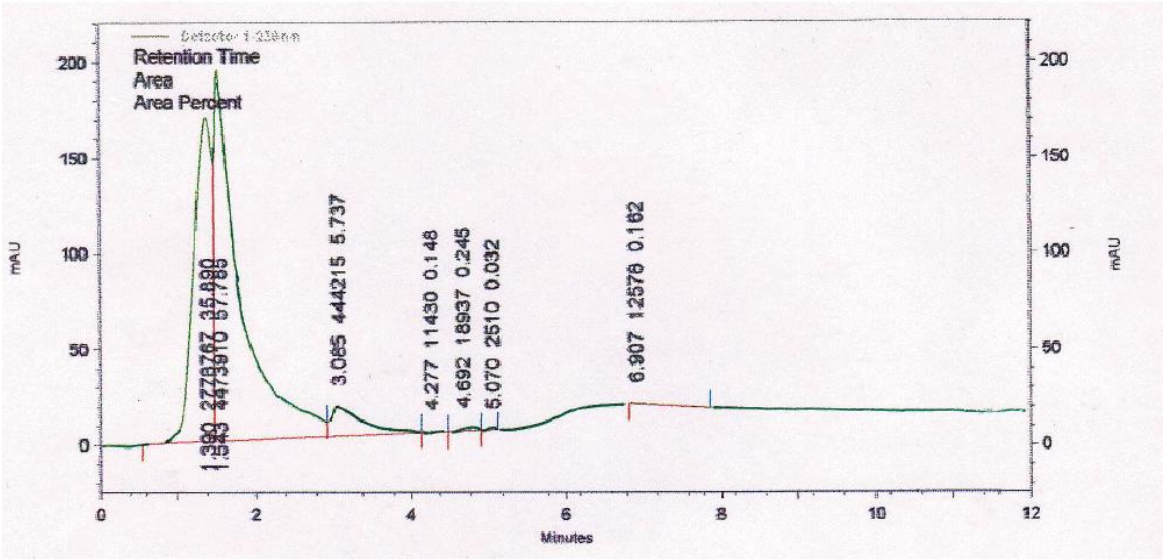
Sample ID: METFHCL 50PPM
PRODUCT: Metformin HCl
W LENGTH: 236 nm
Injection vol. : 20 uL



Sample ID: METFHCL 25PPM
PRODUCT: Metformin HCL
W LENGTH: 236 nm
Injection vol. : 20 uL



Sample ID: ST-1
PRODUCT: Metformin HCL
W LENGTH: 236 nm
Injection vol. : 20 uL



V. RESULTS & DISCUSSION

	Common Name	Botanical name	Family	% MF
	Paneer seed	Withania Coagulens	Solanaceae	57.79

The findings reveal that Withania coagulens(Seeds) show anti hyperglycemic activity due to the probable presence of Metformin hydrochloride which might be the active principle. The result was quite encouraging as (57.79% of Metformin hydrochloride). There is no doubt, if the process of drug development is achieved for Withania coagulens-Seed, a new therapy for diabetes from non- conventional sources will be established. The test sample may be analyzed in detail further.At the same time the environment friendly approach to procure these samples is also encouraging. There will not be any threat to environment.

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